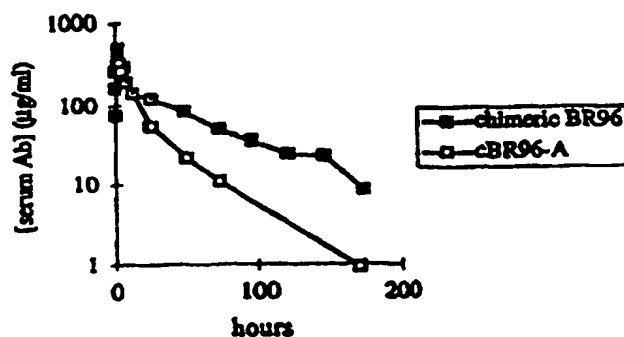




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification⁶ : C12N 15/62, A61K 39/395, 38/17, 47/48, 51/10, C07K 16/30, 16/46, 16/00, C12N 15/13, 1/21, 5/10 // C07K 19/00</p>	A1	<p>(11) International Publication Number: WO 98/05787</p> <p>(43) International Publication Date: 12 February 1998 (12.02.98)</p>
<p>(21) International Application Number: PCT/US97/13562</p> <p>(22) International Filing Date: 1 August 1997 (01.08.97)</p> <p>(30) Priority Data: 60/023,033 2 August 1996 (02.08.96) US</p> <p>(71) Applicant: BRISTOL-MYERS SQUIBB COMPANY [US/US]; 345 Park Avenue, New York, NY 10154 (US).</p> <p>(72) Inventors: ROSOK, Mac, Joanne; 6340 N.E. 194th Street, Seattle, WA 98155 (US). YELTON, Dale, E.; 2307 19th Avenue East, Seattle, WA 98112 (US).</p> <p>(74) Agent: ADRIANO, Sarah, B.; Merchant, Gould, Smith, Edell, Welter & Schmidt, Suite 400, 11150 Santa Monica Boulevard, Los Angeles, CA 90025 (US).</p>		<p>(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>

(54) Title: A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS



Plasma clearance in high LeY expressing dogs chimeric versus constant region mutant of cBR96-2.

(57) Abstract

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an immunoglobulin or Ig fusion protein molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by inactivation of at least a portion of the constant region.

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INTERNATIONAL SEARCH REPORT

Intern: al Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/62 A61K39/395 A61K38/17 A61K47/48 A61K51/10
C07K16/30 C07K16/46 C07K16/00 C12N15/13 C12N1/21
C12N5/10 //C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. GILLIES ET AL.: "Antigen binding and biological activities of engineered mutant chimeric antibodies with human tumor specificities." HUMAN ANTIBODIES AND HYBRIDOMAS, vol. 1, no. 1, 1990, STONEHAM, MA, USA, pages 47-54, XP002050448 see the whole document --- -/--	1-8, 23-25

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. SCHREIBER ET AL.: "An unmodified anticarcinoma antibody, BR96, localizes to and inhibits the outgrowth of human tumors in nude mice." CANCER RESEARCH, vol. 52, no. 12, 15 June 1992, BALTIMORE, MD, USA, pages 3262-3266, XP002050449 see abstract	33,35,36
A	---	1,2,5,7, 8,11-18, 23
A	A. DUNCAN ET AL.: "The binding site for Clq on IgG." NATURE, vol. 332, no. 6166, 21 April 1988, LONDON, GB, pages 738-740, XP002050450 cited in the application see the whole document	1,2,5,7, 8
A	---	1,2,5,7, 8
A	J. LUND ET AL.: "Human FcγRI and FcγRII interact with distinct but overlapping sites on human IgG." THE JOURNAL OF IMMUNOLOGY, vol. 147, no. 8, 15 October 1991, BALTIMORE, MD, USA, pages 2657-2662, XP002050451 cited in the application see abstract	1,2,5,7, 8
A	---	1-8
A	Y. XU ET AL.: "Residue at position 331 in the IgG1 and IgG4 CH2 domains contributes to their differential ability to bind and activate complement." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 5, 4 February 1994, BALTIMORE, MD, USA, pages 3469-3474, XP002050452 cited in the application see abstract see discussion	1-8
A	---	1,2,5,7, 8
	T. MICHAELSEN ET AL.: "One disulfide bond in front of the second heavy chain constant region is necessary and sufficient for effector functions of human IgG3 without a genetic hinge." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 91, no. 20, 27 September 1994, WASHINGTON, DC, USA, pages 9243-9247, XP002050453 see the whole document	1,2,5,7, 8

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	L. TAN ET AL.: "Influence of the hinge region on complement activation, Clq binding, and segmental flexibility in chimeric human immunoglobulins." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 87, no. 1, January 1990, WASHINGTON, DC, USA, pages 162-166, XP002050454 see the whole document ---	1-8
A	EP 0 699 756 A (BRISTOL-MYERS SQUIBB COMPANY) 6 March 1996 cited in the application see examples see claims -----	11-18, 23,25, 28,29, 31-52

INTERNATIONAL SEARCH REPORT

International application No
PCT/US 97/13562

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons.

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
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3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

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2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
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Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
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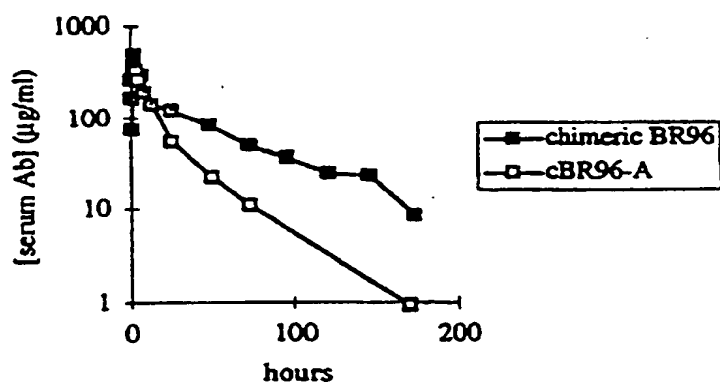
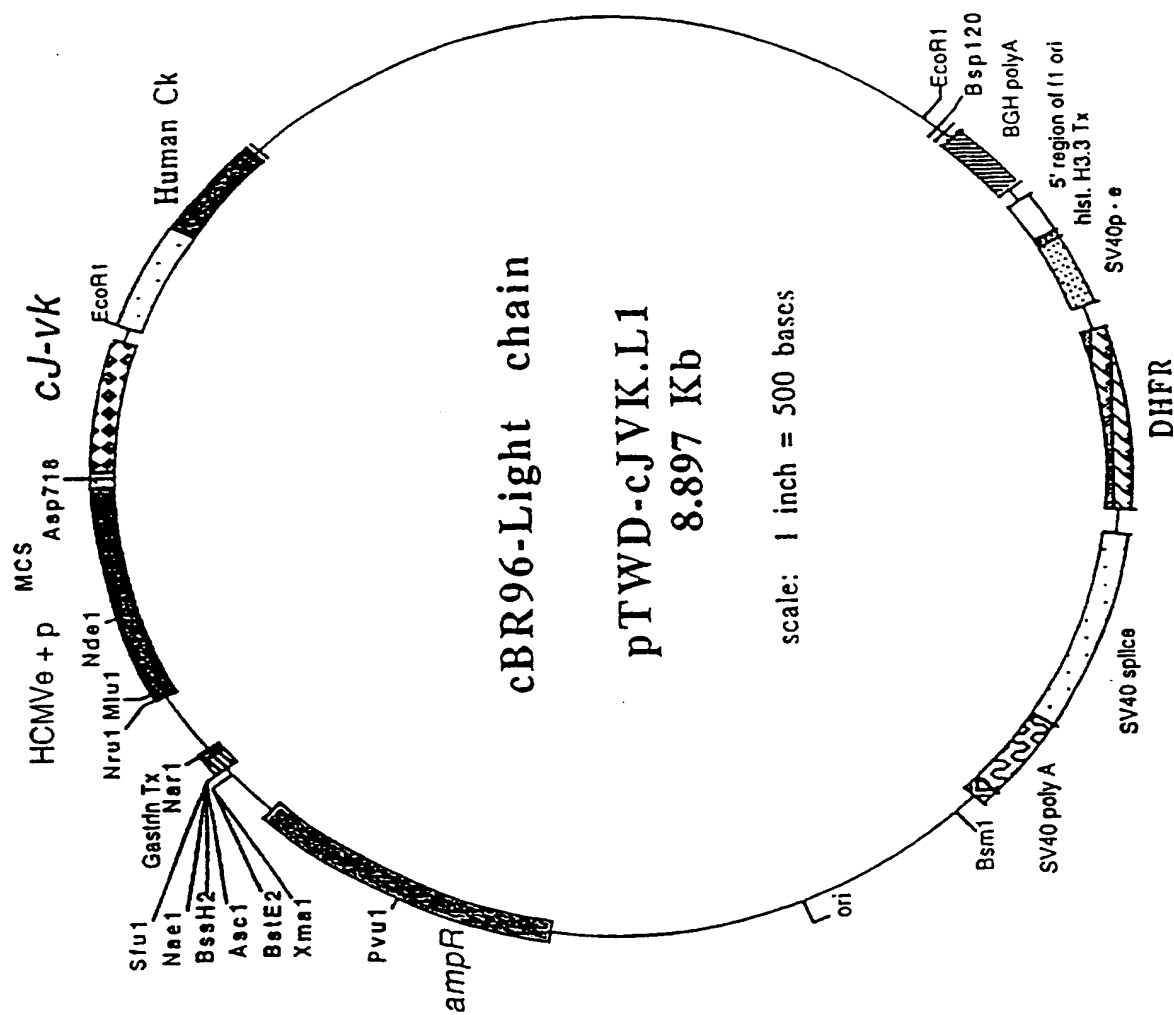


Figure 1. Plasma clearance in high LeY expressing dogs chimeric versus constant region mutant of cBR96-2.

Figure One

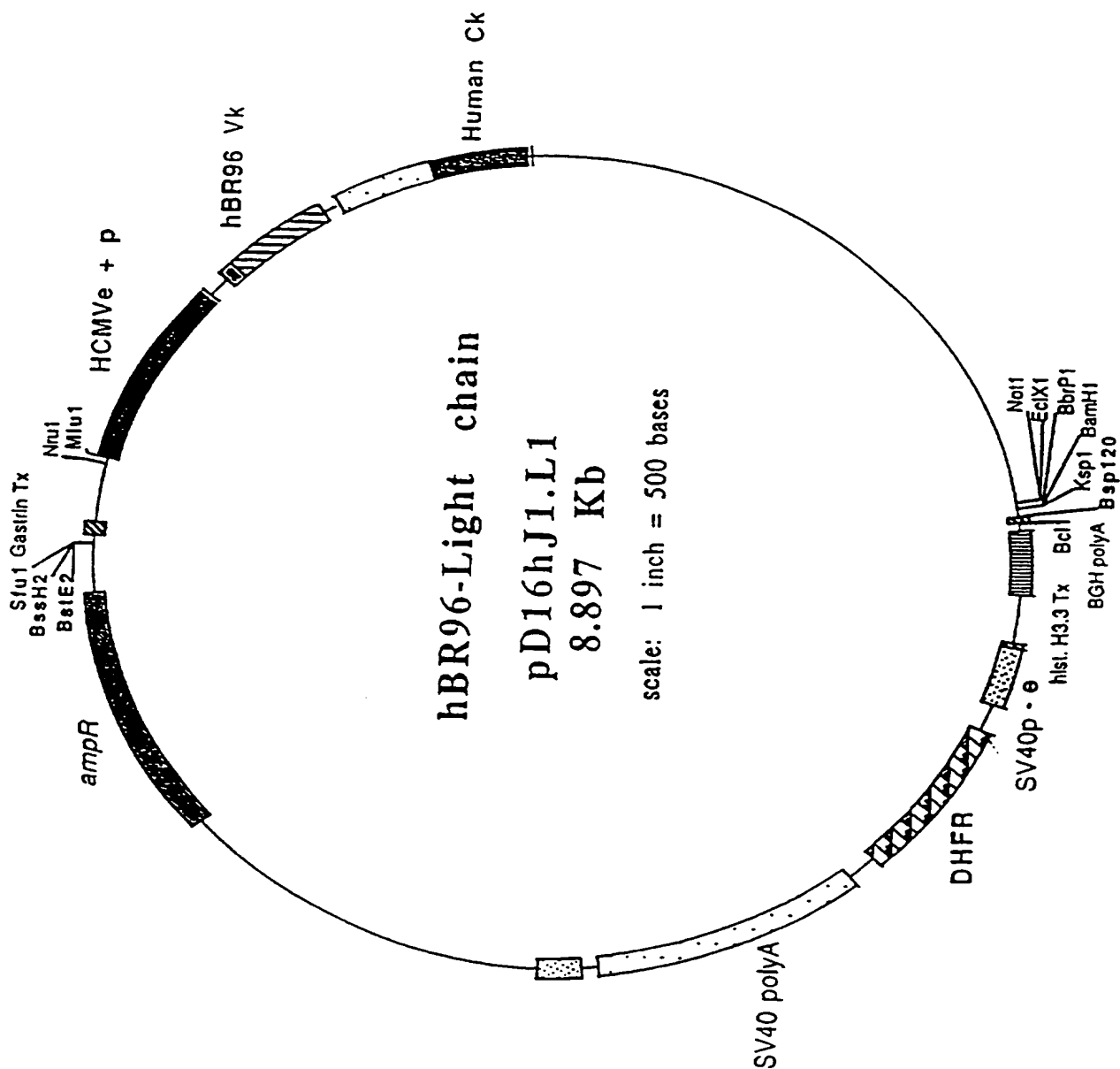
1/56

Figure 2



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Figure 3



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Figure 4

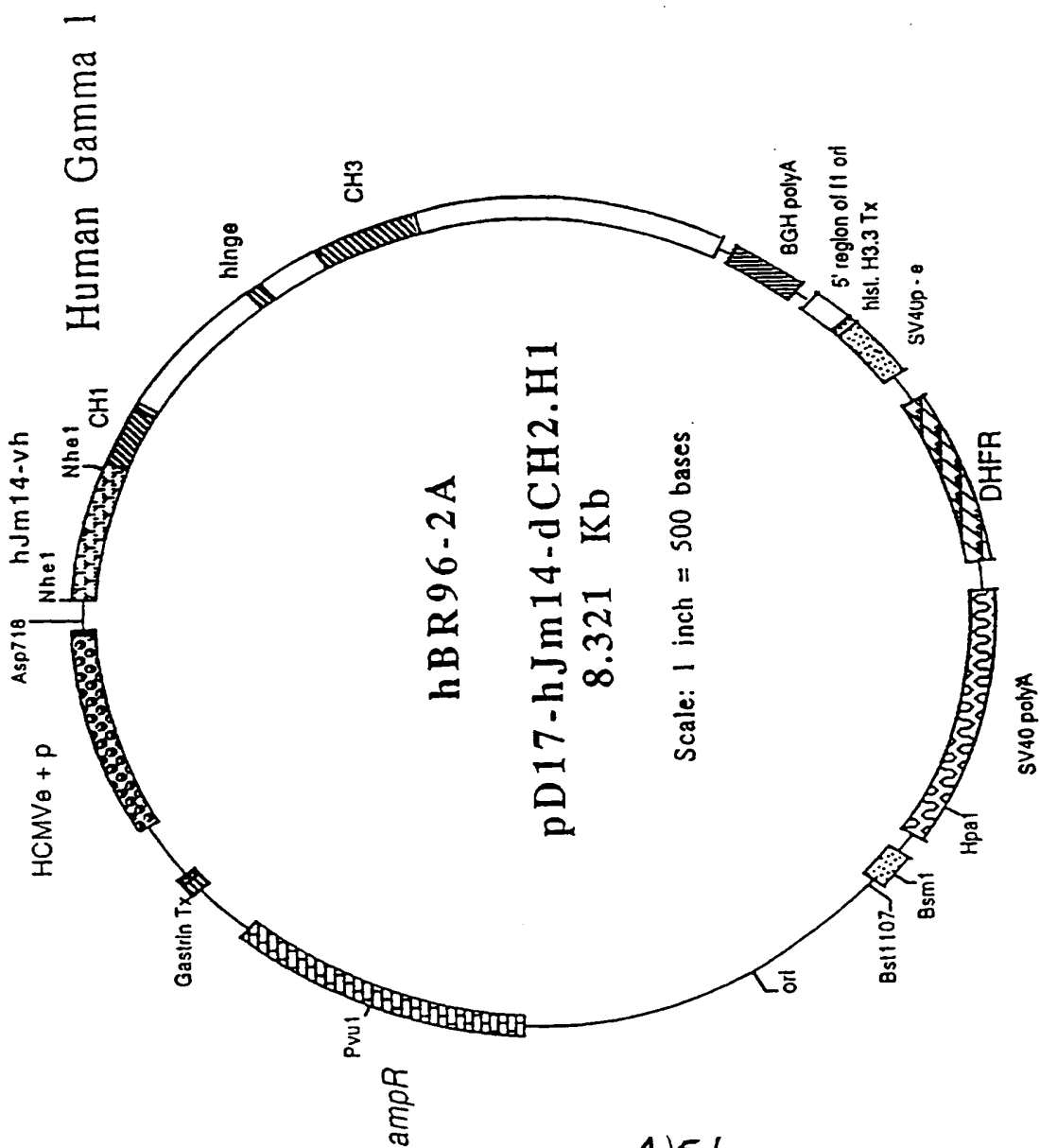
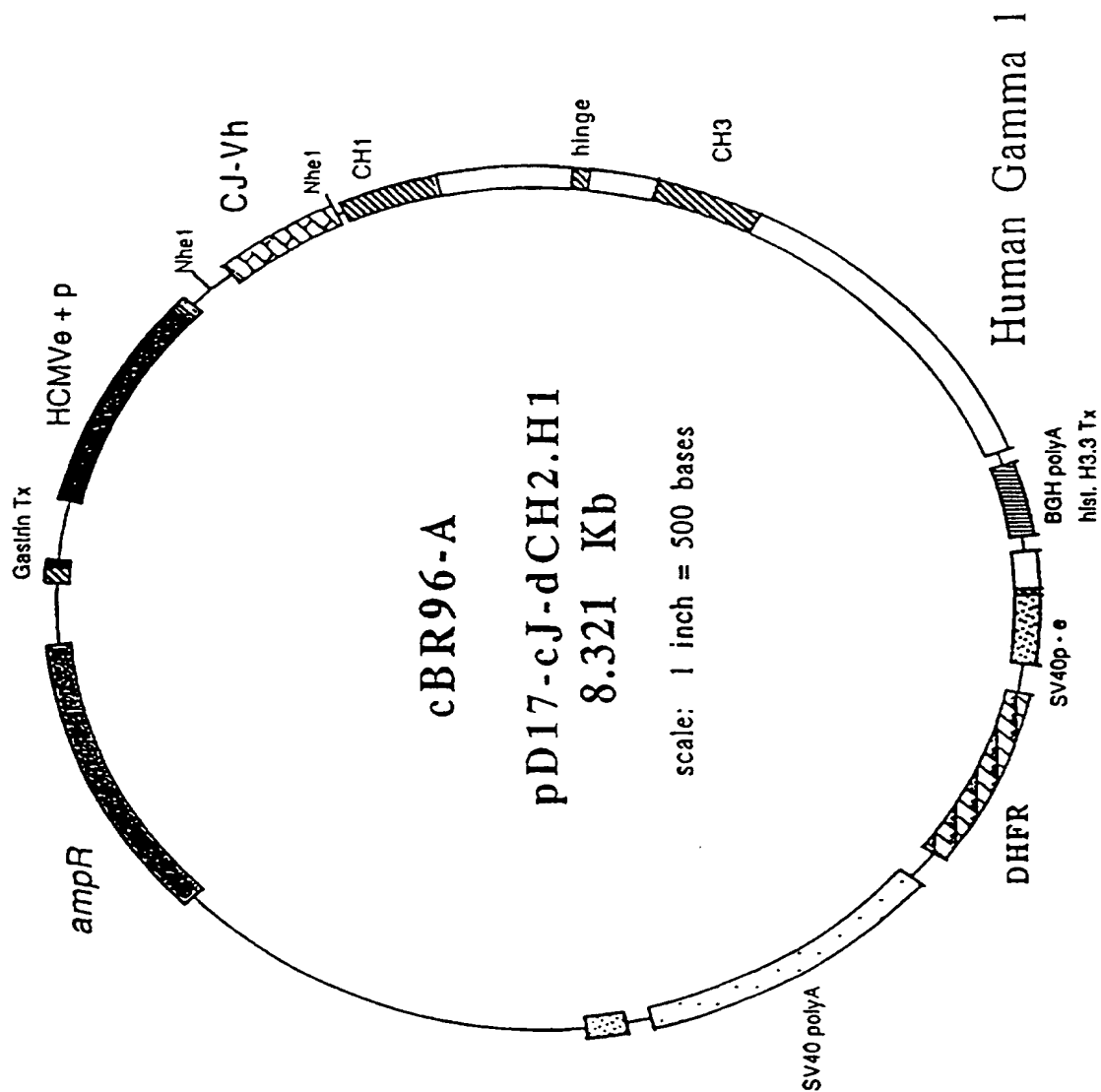
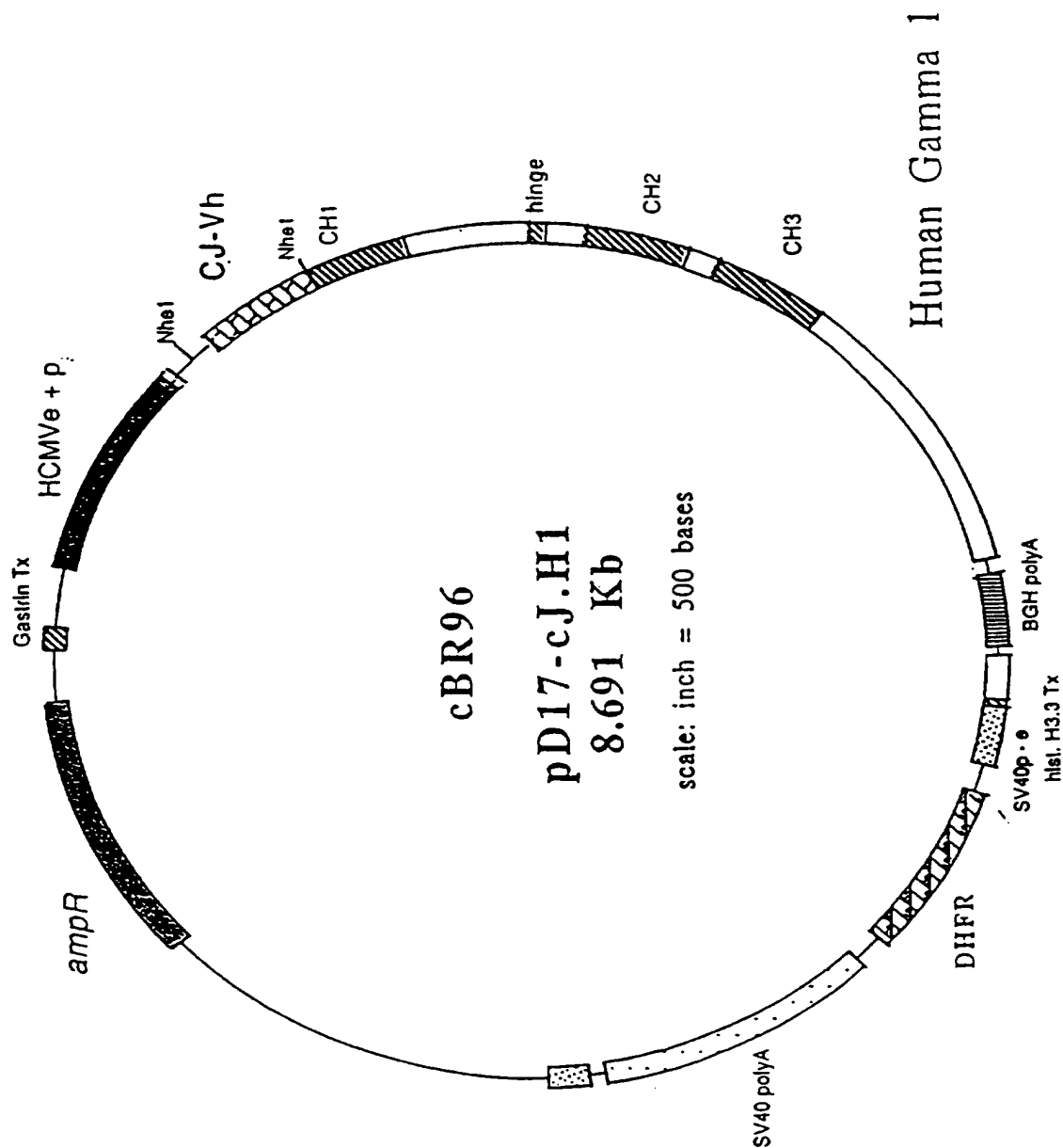


Figure 5



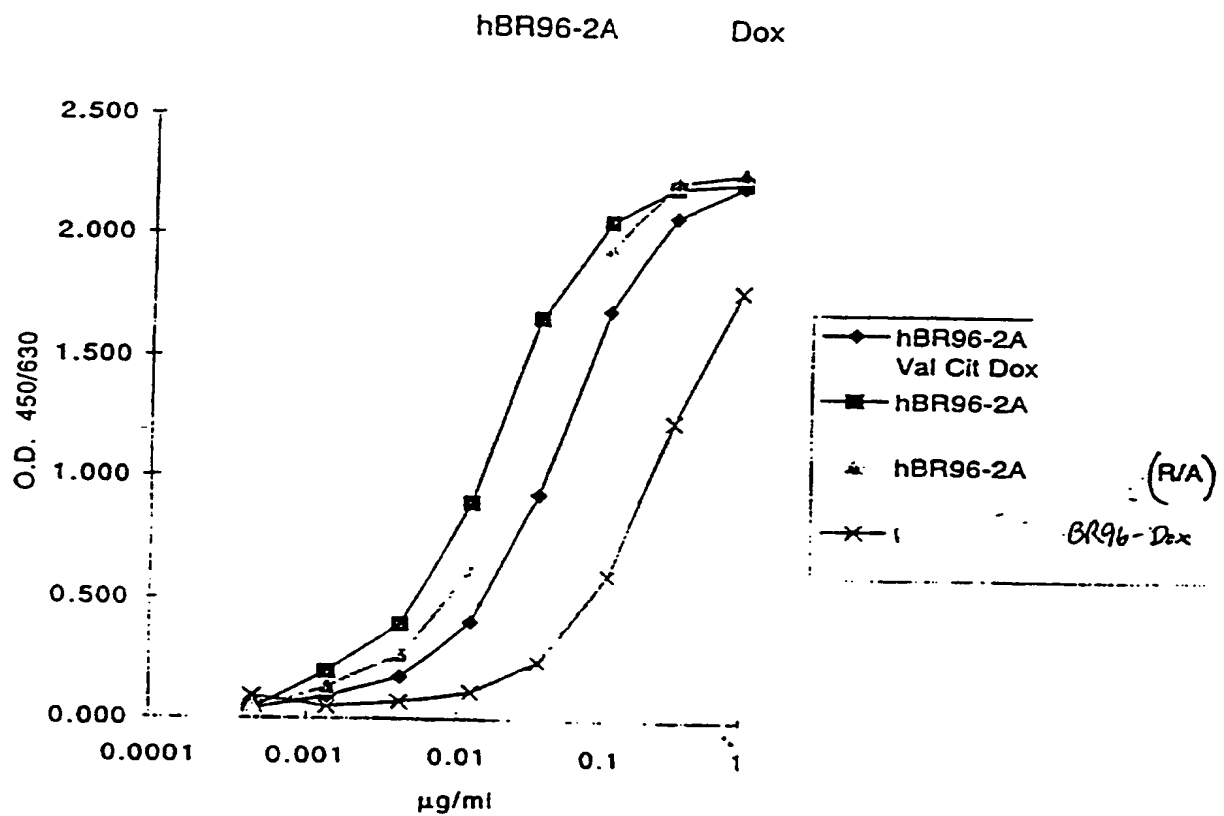
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Figure 6



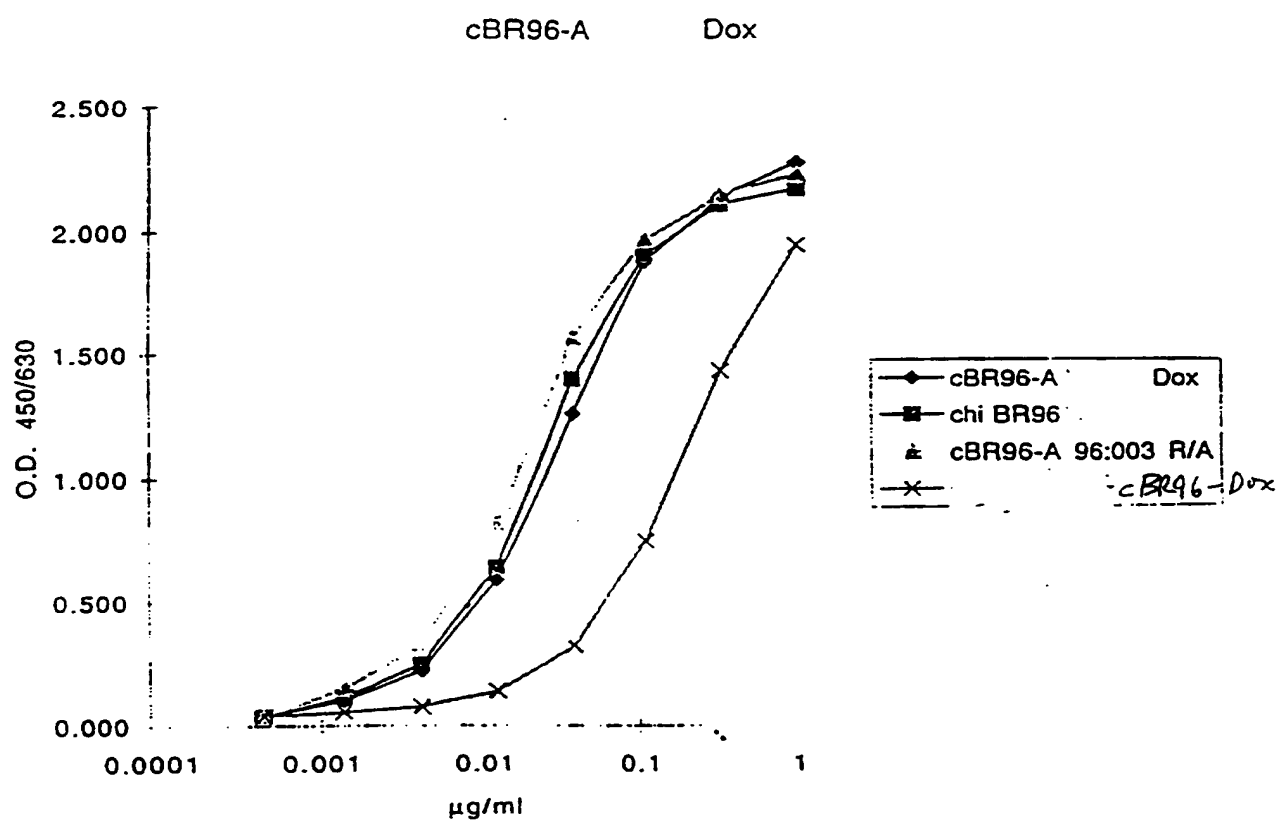
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Figure 7



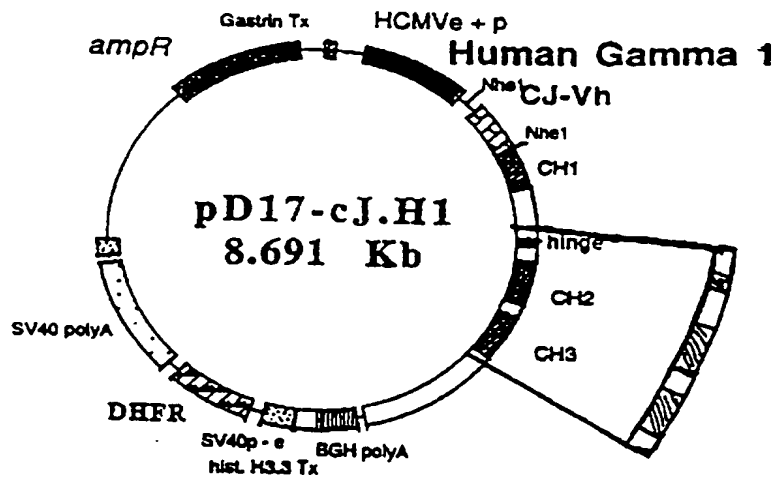
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Figure 8



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A- Hinge + CL + CH3 domains were removed from hR96 IgG1 construct by EcoRI restriction digestion.



B. Hinge + CH3 domains amplified by PCR from L6 IgG1 construct lacking the CH2 domain.

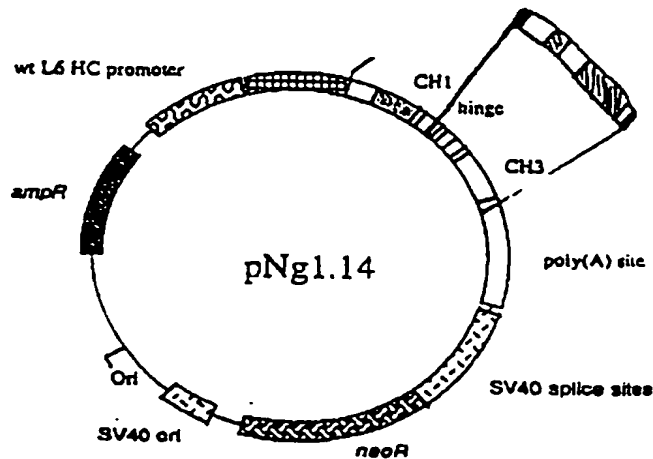


Figure 9

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3 - Hinge + CH3 PCR fragment cloned by homologous recombination into E.co47-III site of BR96 IgG1 molecule.

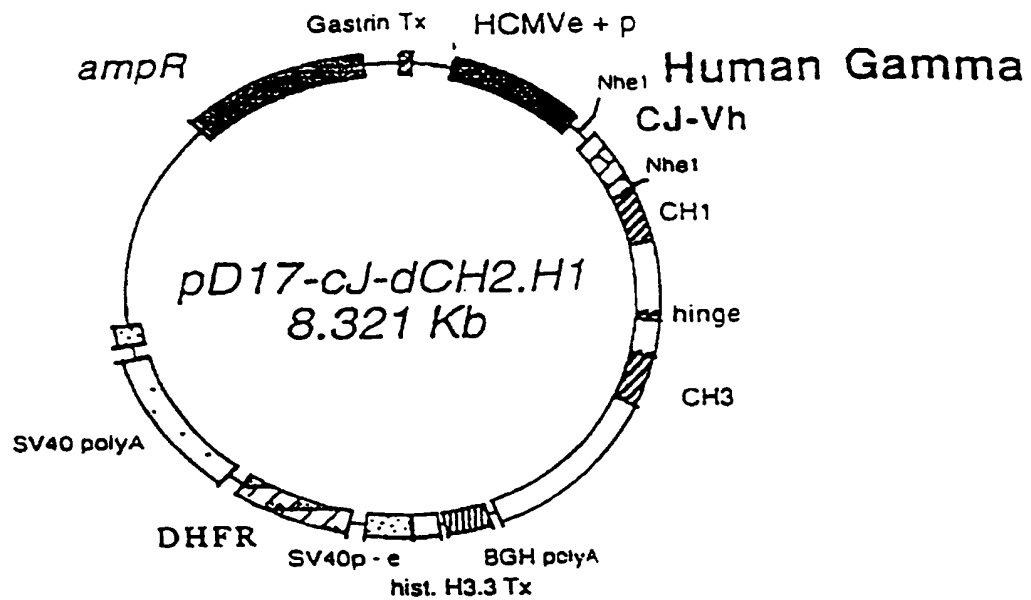


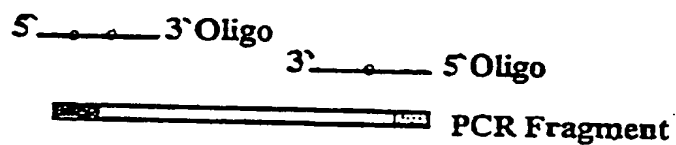
Figure 9

(CONTINUED)

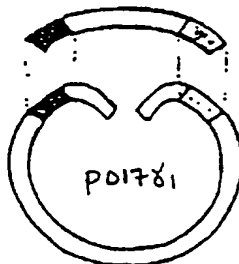
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1- Introduction of mutations by site-directed mutagenesis on double-stranded plasmid DNA.

A- Mutations introduced into synthetic oligonucleotides used for the PCR amplification of CH2 domain.



B- Plasmid DNA linearized inside CH2 domain and co-transformed with PCR fragment into competent DH5 α .



C- Cloning mediated by homologous recombination yields transformants harbouring recombinant plasmids.

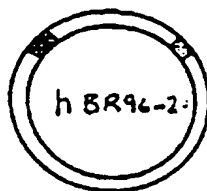
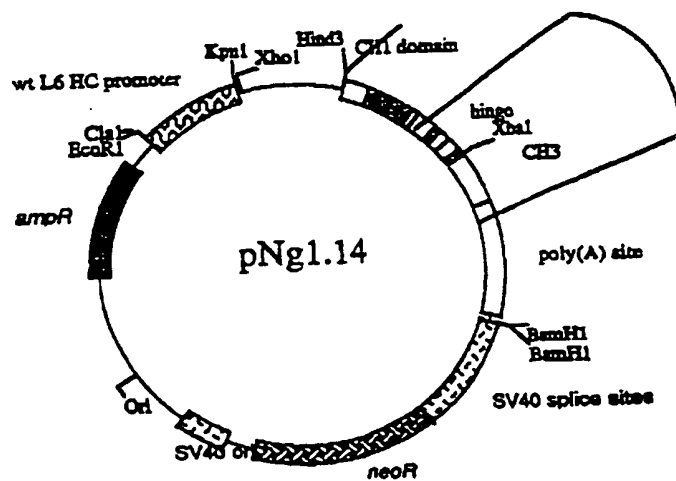


Figure 10

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Figure 11



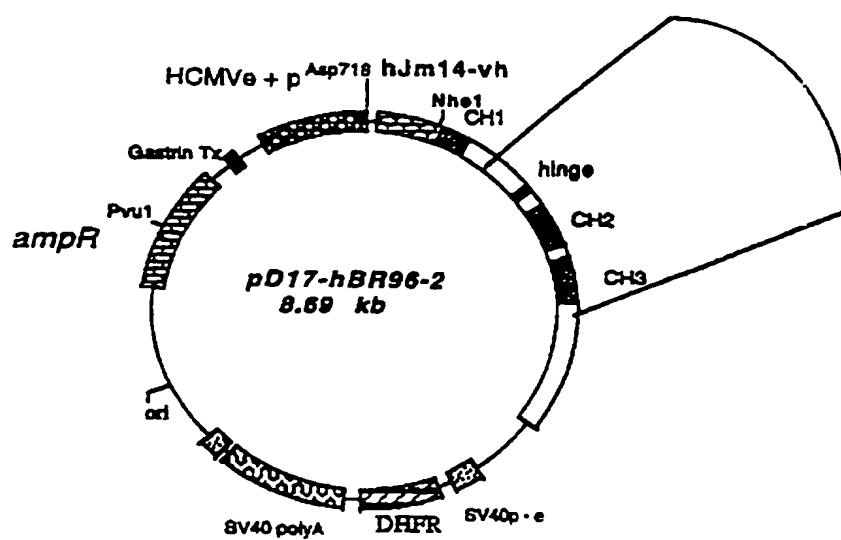


Figure 12

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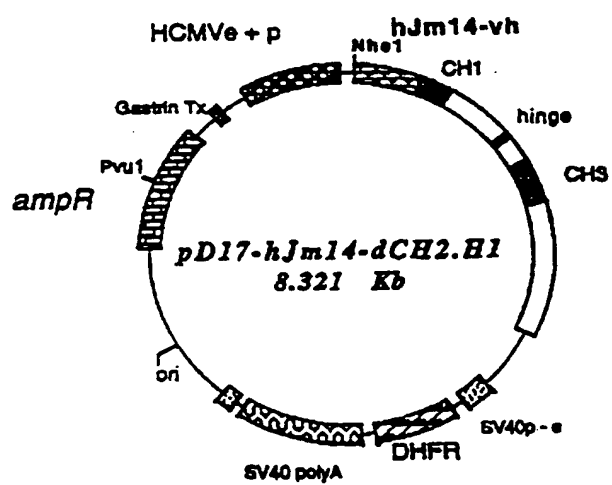


Figure 13

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pD17-cJ-dCH2.H1

10 GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGGCGG GCTTCGAATA GCCAGAGTAA CCTTTTTTTT TAATTTTATT TTATTTTATT 90
 CTGCTTAGCC CTCTAGACGA TCCACTGGAC TCCGCGCGGC CGAAGCTTAT CCGTCTCATT GGAAAAAAA ATTAATAATA NATAAAAATA
 100 TTTGAGATGG AGTTTGGCGC CGATCTCCCG ATCCCTCTATG GTGACTCTC AGTACAACT AGCTATGTC CGCATAGTTA AGCCAGTATC 180
 AAATCTTACC TCAAAACCGC GCTAGAGGGC TAGGGGATAC CAGCTGAGAG TCATGTTAGA CGAGACTACG GCGTATCAAT TCGGTCTATG
 190 TGCCTCCCTG TTTGTGTGTT GAGGTGCTG AGTAGTGGC GAGCAAAAT TAAGCTACAA CAAGGCAAGG CTTCACCGAC AATTGCAATGA 270
 ACGAGGGACG AACACACAC ACCTCCAGGAC TCATCACCGC CTCGTTTTAA ATTCGATGTT GTTCCGTTCC GAACCTGGCT TTAACGTACT
 280 AGAATCTGCT TAGGGTTAGG CGTTTTCGCG TGTCTCGCA TGTACGGGC AGATATACG GTTGACATTT ATTATTGACT AGTTATTATAT 360
 TCTTAGACGA ATCCCAATCC GCANACCGC ACGAAGCGCT ACATGCCCG TCTATATGCG CAATCTGTAC TAATAACTGA TCAATAATTA
 370 AGTAATCAAT TACGGGGTCA TTAGTTTATA GCGCATATAT GGAGTTCCG GTTACATAAC TTACGGTAAA TGCCCGCCTT GGCTGACCGC 450
 TCATTAGTTA ATGCCCCAGT AATCAAGTAT CCGGTATATA CCTCAAGGCG CAATGTATTT AATGCCATTT ACCGGCGGGA CCGACTGGCG
 460 CCAACGACCC CCGCCCATTT AGTCAATAA TGACGTATGT TCCCATAGTA ACGCCATATG GGACTTTCCA TTGACGTCAA TGGGTGGACT 540
 GGTGCTGGG GCGCGGTAC TGCAGTTATT ACTGCATACA AGGATATCAT TGCGGTTATC CCTGAAAGGT AACTGCAGTT ACCCACCTGA
 550 AATTACGGTA AACTGCCAC TTGGCAGTAC ATCAAGTGA TCATATGCCA AGTACGCCC CTATTGAGCT CAATGACGGT AAATGGGCCG 630
 TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGG GATACTGCA GTTACTGCCA TTTACCGGGC
 640 CCTGGCAITTA TGCCCAAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAAT CGCTATTACC ATGCTGATGC 720
 GGACCGTAAT ACGGGTCAAT TACTGGATA CCTGAAAGG ATGAACCGTC ATGTAGATGC ATAAATCAGTA GCGATAATGG TACCACTACG
 730 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGA TTTCCAAATC TCCACCCCAT TGACGTCAAT GGGAGTTTGT 810
 CCAAAACCGT CATGTAGTTA CCGCACCTA TCGCCAACT GAGTGCCCT AAGGTTACG AGGTGGGTA ACTGCAGTTA CCTCAACA
 820 TTTGGCACCA AATCAACCG GACTTTCCAA AATGTCGTAA CAATCCGCC CCATTACGC AATGCGCGG TAGCGTGTGA CGGTGGGAGG 900
 AAACCGTGGT TTTAGTTGCC CTGAAGGTT TTACAGCATT GTTCAGGCGG GGTAACCTGC GTTACCCGCC ATCCGACAT GCCACCTCC

Figure 14

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pD17-cJ-dCH2.H1

910 TCATATTAAG CAGAGCTCTC TGGCTAACTA GAGAACCCAC TGCTTACTGG CTTATCGAAA TTAATACGAC TCACTATAGG GAGACCCNAG 990
 AGATATATTG GTCTCGAGAG ACCGATTGAT CTCCTGGGTG ACGAATGACC GAATAGCTTT AATTATGCTG AGTATATGCC CTCTGGGTTC
 1000 CTTGGTACCA ATTTAAATTTG ATATCTCCTT AGGTCTCGAG TCTCTAGATA ACCGGTCAAT COATTGGAAT TCTTGGGCC GCTTGTCTAGC 1080
 GAACCATGGT TAAATTTAAC TATAGAGGAA TCCAGAGCTC AGAGATCTAT TGGCCAGTTA GCTAACCTTA AGAACGCCGG CGAACGATCG
 1090 CACCATGGAG TTGTGGTTAA GCTTGGTCTT TCCCTGTCTT TGTTTTAAAA GGTGTCCAGT GTGAAGTCAA TCTGGTGGAG TCTGGGGGAG 1170
 GTGGTACCTC AACACCAATT CGAACCCAGGA AGGAACAGGA ACAAAATTTT CCACAGGTCA CACTTCACTT AGACCACCTC AGACCCCTC
 1180 GCTTAGTCCA GCTCTGAGGG TCCCTGAAAG TCTCTGTGTG AACCTCTGGA TTCACCTTCA GTGACTATTA CATGTATTGG GTTCGCCAGA 1260
 CGAATCAGT CGGACCTGCC AGGACTTTC AGAGACACA TTGGAGACCT AAGTGAAAGT CACTGATAAT GTACATAACC CAAGCGTCT
 1270 CTCCAGAGAA GAGGCTGGAG TGGGTGGCAT ACATTAGTCA AGGTGGTGAT ATAACCGACT ATCCAGACAC TGTAAAGGT CGATTCACCA 1350
 GAGGTCTCTT CTCGACCTC ACCCAGCGTA TGTAAATCAGT TCCACCACTA TATTGGCTGA TAGTCTGTG ACATTTCCCA GCTAAGTGT
 1360 TCTCCAGAGA CAATGCCAAG AACACCTCTGT ACCTGCAAT GAGCGTCTG AAGTCTGAGG ACACAGCCAT GTATTACTGT GCAAGAGGCC 1440
 AGAGGTCTCT GTTACGGTTC TTGTGGGACA TGGACGTTTA CTCGCGAGAC TTCAGACTCC TGTGTGGTA CATAATGACA CGTTCCTCCG
 1450 TGGACGAGG GGCCTGGTTT GCTTACTGGG GCAAGGGAC TCTGGTCAAG GTCTCTGTAG CTAGCACCAA GGGCCCATCG GTCTTCCCCC 1530
 ACCTGCTGCC CCGGACCAA CGAATGACCC CGCTTCCCTG AGACCACTGC CAGAGACATC GATCGTGGT CCCGGGTAGC CAGAACGGGG
 1540 TGGCACCTC CTCGAGAGC ACCTCTGGG GCACAGCGC CCTGGGCTGC CTGGTCAAG ACTACTTCC CGAACCGGTG ACGGTGTCTG 1620
 ACCGTGGAG GAGGTCTCG TGGAGACCC CGTGTCCCG GACCCGAGC GACCAGTCC TGATGAAGG GCTTGGCCAC TGCCACAGCA
 1630 GGAATCAGG CGCCCTGACC AGCGGCTGC ACACCTTCCC GGCTGTCTTA CAGTCTCTAG GACTTACTC CCTCAGCAGC GTGTCTACCG 1710
 CCTTGAGTCC CGGGACTGG TCGCCGACG TGTGGAAGG CCACAGGAT GTCAGGATC CTGAGATGAG GGAGTCTGCG CACCAGTGGC
 1720 TGCCCTCCAG CAGCTTGGG ACCCAGACCT ACATCTGCA CGTGAATCAC AAGCCAGCA ACACCAAGT GGACAAGAA GTTGTGAGA 1800
 ACGGAGGTC GTCGAACCCG TGGGTCTGGA TGTAGACGTT GCACTTAGTG TTCGGGTCTT TGTGTCTT CAACCACTCT

Figure 14
(Continued)

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pD17-cJ-dCH2.H1

1810	1820	1830	1840	1850	1860	1870	1880	1890
GCCAGCACA	GGGAGGAGG	GTGTCGTCTG	GAAGCCAGGC	TCAGCGCTCC	TGCTTGACG	CATCCCGGCT	ATGCAGCCCC	AGTCCAGGGC
CGGTCTGTGT	CCCTCCCTCC	CACAGACGAC	CTTCGGTCCG	AGTCGGAGG	ACGGACCTGC	GTAGGGCCGA	TACGTCCGGG	TCAGTCCCG
1900	1910	1920	1930	1940	1950	1960	1970	1980
AGCAAGGCAG	GCCCGTCTG	CCTCTTCACC	CGGAGGCCCTC	TGCCCGCCCC	ACTCATGCTC	AGGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG
TCGTTCCTGC	CGGGCAGAC	GGAGNAGTGG	GCCTCCGGAG	ACGGCGGGG	TGAGTACGAG	TCCCTCTCCC	AGNAGACCGA	AAAGGGGTC
1990	2000	2010	2020	2030	2040	2050	2060	2070
GCTCTGGCA	GGCAGAGGT	AGGTGCCCCCT	AACCCAGGCC	CTGCACACAA	AGGGCAGGT	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC
CGAGACCCGT	CCGTCTCCGA	TCCACGGGA	TTGGGTCCCG	GACGTGTGTT	TCCCCGTCCA	CGACCCGAGT	CTGGACGGTT	CTCGGTATAG
2080	2090	2100	2110	2120	2130	2140	2150	2160
CGGGAGGACC	CTGCCCTCTGA	CCTNAGCCCA	CCCCAAAGGC	CNAACTCTCC	ACTCCCTCAG	CTCGGACACC	TCTCTCTCCTC	CCAGATTCCA
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2170	2180	2190	2200	2210	2220	2230	2240	2250
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CATTAGGGT	TAGNAGAGAG	ACGTCTCGGG	TTTAGNACAC	TGTTTGTAGT	GTGTACGGGT	GGCACGGGTC	CATTGGTTCG	GGTCCGGAGC
2260	2270	2280	2290	2300	2310	2320	2330	2340
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GGGAGGTCTGA	GTTCGGCCCT	GTCCACGGGA	TCTCATCCGA	CGTAGGTTCC	TGTGTGGTGC	ACCCATGGTT	GTACAGGCCCT	CGGTGTACCT
2350	2360	2370	2380	2390	2400	2410	2420	2430
CAGAGGCCGG	CTCGGCCAC	CCCTCTGCCCT	GAGAGTGACC	GCTGTACCNA	CCTCTGTCCC	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA
GTCTCCGGCC	GAGCCGGTG	GGAGACGGGA	CTCTCACTGG	CGACATGGTT	GGAGACAGGG	ATGTCCCCTC	GGGGCTCTTG	GTGTCCACAT
2440	2450	2460	2470	2480	2490	2500	2510	2520
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GTGGGACGGG	GGTAGGGCCC	TACTCGACTG	GTCTTTGGTC	CAGTCCGACT	GGACGGACCA	GTTTCCGAG	ATAGGGTCCG	TGTAGCGGCA
2530	2540	2550	2560	2570	2580	2590	2600	2610
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CCTCACCCCTC	TCGTATACCCG	TCGGCCCTCT	GTTGATGTTT	TGGTGGGAG	GGCAGCACCT	GAGGCTCCG	AGGAAGAAGG	AGATGTCGTT
2620	2630	2640	2650	2660	2670	2680	2690	2700
GCTCACCCGTG	GACAAGAGCA	GGTGGCAGCA	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	ACACGCAGAA
CGATGGGCAC	CTGTTCTCGT	CCACCCCTCGT	CCCTTGCAG	ANAGTACGA	GGCACTACCT	ACTCCGAGAC	GTCTTGGTGA	TGTGCGTCTT

Figure 14
(continued)

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2710 GAGCCTCTCC CTGTCTCCGG GTAAATGAGT GCGAGCGCGG GCAAGCCCC GCTCCCGGG CTCTCGGGT CGACACGAGG TCCTTGGCAC 2790
 CTGGAGAGG GACAGAGGCC CATTTACTCA CGCTGCCGGC CGTTCCGGGG CGAGGGGCC GAGAGGCCA GCGTGTCTCT ACGAACCGTG
 2800 GTACCCCTCG TACATACTTC CCGGGCGCC AGCATGMAA TAAAGCACCC AGCGTCCCG TGGGCCCTCG CGAGACTGTG ATGTTCTTT
 CATGGGGGAC ATGTATGAAG GGGCCGGGG TCGTACTTTT ATTTCTGGG TCGGACGGG ACCCGGGAC GCTCTGACAC TACCAAGAAA 2880
 2890 CCACGGTCA GGGGAGTCT GAGGCTGAG TGGCATGAGG GAGGAGAGC GGTTCACCT GTCCCCACAC TCGCCACAGC TGTGCAGGTG 2970
 GGTGCCAGT CCGCTCAGA CTCGCTCAG ACCGTACTCC CTCGCTCTCG CCCAAGTGA CAGGGGTGTG ACCGGGTCCG ACACGTCCAC
 2980 TGCCTGGCC CCTTAGGGTG GGGCTCAGCC AGGGCTGCC CTCTGGCAGG TGGGGGATTT GCCAGCTGTG CCTCCCTCC AGCAGCACCT 3060
 ACGGACCCGG GGGATCCAC CCGGATCCG TCCCGGACGG GAGCGTCC ACCCCCTAA CCGTCCGACC GGGAGGGAGG TCGTCTGTGA
 3070 GCGCTGGCT GGGCCACGG AAGCCCTAGG AGCCCTGGG GACAGACACA CAGCCCTCG CTCTGTAGGA GACTGTCTTG TTCTGTGAGC 3150
 CCGGACCCGA CCGGTGCC CTCTGGATCC TCGGGGACCC CTGTCTGTGT GTCTGGGACG GAGACATCT CTGACAGGAC AAGACACTCG
 3160 GCGCTGTCC TCCCGACCT CATGCCACT CCGGGGCTG CCTAGTCCAT GTGCGTAGGG ACAGGCCCTC CCTCACCCAT CTACCCCCAC 3240
 CCGGACAGG AGGCTGAG GTACGGGTGA GCGGCTGAC GCGCCCTGAC GGATCAGGTA CACGCATCCC TGTCGGGAG GGAGTGGTA GATGGGGTG
 3250 GGCCTAAC CCTGGCTGCC CTGCCAGCC TCGCACCCG ATGGGACAC AACGACTCC GGGGACATGC ACTCTCGGG CCTGTGGAGG 3330
 CCGTGATTG GACCCGACG GACGGGTGG AGCGTGGCG TACCCCTGTG TTGGCTGAGG CCCCTGTACG TGAGAGCCCG GACACCTCC
 3340 GACTGTGCA GATGCCACA CACACACTCA GCGCAGACC GTTCACAAA CCGCGACTG AGGTTGGCG GCCACAGGC CACCACAC 3420
 CTGACCACGT CTACGGGTGT GTGTGTAGT CCGGTCTGG CAAGTGTGT GGGCGTGAC TCCAACCCGG CGGTGTCCG GTGGTGTG
 3430 ACAGTGCAC GCTCACA CCGAGCTCA CCGGGCGAA CTGCACAGCA CCCAGACCAG AGCAAGTCC TCGCACAGT GAACACTCT 3510
 TGTGCAGTG CGAGTGTGT GCTCTGGAGT GGGCCCGCTT GACGTGTCT GGGTCTGGTC TCGTTCCAG AGCGTGTGA CTTGTGAGGA
 3520 CGGACACAG CCCCCAGG CCGCAGGG CACCTCAGG CCGCAGGCC TCTCGGCG TCTCCACAT TTCTCCACAT GCTGACCTGC TCAGACAAAC 3600
 GCTGTGTCC GGGGTGCTC GGGTGTGCG GTGGAGTTCC GGTGTCTCG AGAGCCGTG AAGAGGTGA CGACTGGAC AGTCTGTTG

Figure 14
(continued)

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3610 CCAGCCCTCC TCTCACAAGG GTGCCCCGTC AGCGCCACACA CACACACAGG GGATCACAACA CCAGTCAACG TCCCTGGCCC TGCCCCACTT 3690
 3700 GGTGCGGAGG AGAGTGTCTC CACGCGGACG TCGGGGGTGT GTGTGTGTCTC CTTAGTGTGT GGTGACGTGC AGGACCGGG ACCGGGTGAA 3780
 3790 CCAAGTCCG CCTTCCCTG CAGGACGGAT CAGCCTCGAC TGTGCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCTCC CCGGTGCTT 3870
 3800 GGGTACGGC GGAAGGGAC GTCTGCTTA GTGAGCTG GTGAGCTG ACACGGAAGA TCAACGGTGT GTAGACAACA AACGGGAGG GGGCACGAA 3960
 3810 CATTGACCT GGAAGGTGC ACTCCACTG TCCCTTCTTA ATAAATGAG GAAATTGCAT CGCATTTCT GAGTAGTGT CATCTATTTC 3870
 3820 GGAAGTGGG CCTTCCACGG TGAGGGTAC AGGAAGGAT TATTTTACTC CTTTAACTTA GCGTAACAGA CTATCCACA GTAAGATAAG 3960
 3830 TGGGGGGTG GGTGGGGCAG GACAGCAGG GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGCTCT ATGGCTTCTG 3950
 3840 ACCCCCCACC CCACCCCGTC CTGTGCTTCC CCGCTCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTACG CCACCCGAGA TACCGAAGAC 3960
 3850 AGCGGGAAG AACCACTGG GGTCTAGGG GGTATCCCA CGGCCCTGT AGCGGGCAT TAAGCGCGC GGTGTGGTG GTTACGGCA 4050
 3860 TCGCCCTTC TTGCTGACC CCGAGATCCC CCATAGGGT GCGCGGACA TCGCCGCTTA TCGCCGCTTA ATTGCGCGC CCCACACC CAATGCGGT 4140
 3870 GCGTACCGC TACACTTGC AGCGCCCTAG CGCCCGCTCC CGCCCGCTCC TTTGCTTTC TTTCCCTCT TTTCTGCCAC GTTCGCGGG CCTCTCAAA 4140
 3880 CGCACTGGG ATGTGACGG TCGCGGGATC GCGGCGAGG AAGCGGAG AAGCGAAAG AAGGAAAGGA AAGAGCGGTG CAAGCGGCC CGAGCTTTT 4230
 3890 AAGGGAAGA AAGCATGCAT CTCAATTAGT CAGCAACCAT AGTCCGCCC CTAACCTCG CCTAACTCCG CCCAGTTCG 4320
 3900 TTCCCTTTT TCTGACGTA GAGTTAATCA GTCGTTGTA TCAGGCGGG GATTGAGCG GATTGAGCG GATTGAGCG GGTCAAGC 4320
 3910 CCCATTCTC GCCCATGGC TGACTAATTT TTTTATTTA TGCAGAGGC GAGGCCGCT CGGCTCTGA GCTATTCCAG AAGTAGTGAG 4320
 3920 GGGTAAGAG CCGGTACCG ACTGATTAA AAAATTAAT ACGTCTCGG CTCCGGCGGA GCGGAGACT CGATAAGGT TTCATCACTC 4410
 3930 GAGGCTTTT TGGAGGCCA GGTCTTTCG AAGACTTGG ACAGTTCAG GCTCGATT TCGCGCAAT TTAGCGCAA TCCTAGCGTG 4410
 3940 CTCCGAAAA ACCTCCGGT CCGAAACGT TTTTCGAAC TGTGAGTCC CGACGCTAA CGCGGTTG AACTGCGGT AGGATCGAC 4500
 3950 AAGGCTGTA GGTATTTATC CCGCTGCCA TCATGTTTG ACCATTGAAC TGCATCGTC CCGTGTCCA AATATGGG ATTGGCAGA 4500
 3960 TTCCGACCAT CCTAAATAG GGGGACGGT AGTACCAAG TGGTAACCTG TGGTAACCTG TGGTAACCTG TGGTAACCTG TGGTAACCTG 4500

Figure 14
(continued)

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4510 ACGGAGACCT ACCCTGGCCT CCGCTCAGGA ACAGATTCAA 4540 4550 4560 4570 4580 4590
 TCCCTCTGGA TGGGACCGGA GCGAGTCCT TGCTCAAGTT CATGAAGGTT TCCTACTGGT GTTGGAGAAG TCACCTTCCA TTTGTCTTAG
 4600 TGGTGATTAT GGGTAGGAAA ACCTGGTTCT CCATTCTCGA GAAGATCGA CCTTTAAAGG ACAGAAATAA TATAGTTCTC AGTAGAGAAC
 ACCACTAATA CCCATCCCTT TGGACCAAGA GGTAAGGACT CTTCTTAGCT GGAAATTTCC TGCTTTAAAT ATATCAAGAG TCATCTCTTG
 4690 4700 4710 4720 4730 4740 4750 4760 4770
 TCAAGAAC ACCACGAGGA GCTCATTTTC TTGCAAAAG TTTGGATGAT GCCTTAAGAC TTATTGAACA ACCGGAATTG GCAAGTAAG
 AGTTTCTGG TGGTGCTCCT CGAGTAAAG AACGGTTTTC AACCTACTA CGGAATCTG AATAACTTGT TGGCCTTAAC CGTTCAATTC
 4780 4790 4800 4810 4820 4830 4840 4850 4860
 TAGACATGGT TTGGATAGTC GGAGGCAGTT CTGTTTACCA GGAAGCCATG AATCAACCCAG GCCACCTTAG ACTCTTTTGT ACAAGGATCA
 ATCTGTACCA AACCTATCAG CCTCCGTCAA GACAAATGGT CCTTCGGTAC TTAGTTGGTC CGTGGAAATC TGAGAAACAC TGTTCCTAGT
 4870 4880 4890 4900 4910 4920 4930 4940 4950
 TGCAGGAAT TTAAAGTGAC ACCTTTTTC CAGAAATGA TTTGGGAAA TATAAACTTC TCCAGAAATA CCCAGCGTC CTCTCTGAGG
 ACGTCTTAA ACTTTCAC TGCAAAAAGG GTCTTAACT AAACCCCTTT ATATTTGAAG AGGTCTTAT GGTCCCGCAG GAGAGACTCC
 4960 4970 4980 4990 5000 5010 5020 5030 5040
 TCCAGGAGGA AAAAGGCATC AAGTATAGT TTGAAGTCTA CGAGAAGAAA GACTAACAGG AAGATGCTTT CAAGTTCTCT GCTCCCTCC
 AGTCTCTCT TTTCCGCTAG TTCATATTTCA AACCTCAGAT GCTCTTCTTT CTGATTGTCC TTCTACGAAA GTTCAAGAGA CGAGGGAGG
 5050 5060 5070 5080 5090 5100 5110 5120 5130
 TAAAGCTATG CATTTTATA AGACCATGGG ACTTTTGTG GCTTTAGATC TCCTTTGTAA GGAACCTTAC TTCTGTGTG TGACATAAT
 ATTCTGATAC GTAAAATAT TCTGGTACCC TGAACACGAC CGAAATCTAG AGAAACACTT CCTTGGAAATG AAGACACCCAC ACTGTATTAA
 5140 5150 5160 5170 5180 5190 5200 5210 5220
 GGACAACTA CCTACAGAGA TTTAAAGCTC TAAGGTAAAT ATAAATTTT TAAAGTGATA ATGTGTAAA CTACTGATTC TAATGTGTTG
 CCTGTTTGT GGTGTCCT AAATTTGAG ATTCCATTTA TATTTTAAA ATTCAATAT TACACATAT TATGACTAAG ATTAACAAAC
 5230 5240 5250 5260 5270 5280 5290 5300 5310
 TGTATTTTAG ATTCCAACCT ATGGAACCTGA TGAATGGGAG CAGTGTGGA ATGCCTTTAA TGAGGAAAC CTGTTTTGCT CAGAAGAAAT
 ACATATAATC TAAGGTGGA TACCTTGACT ACTTACCCTC GTACACACCT TACGGAATTT ACTCTTTTG GACAAAACGA GTCTCTTTA
 5320 5330 5340 5350 5360 5370 5380 5390 5400
 GCCATCTAGT GATGATGAGG CTACTGCTGA CTCTCAACAT TCTACTCCTC CAATAAAGAA GAGAAAGTA GAAGACCCCA AGGACTTTCC
 CGGTAGATCA CTACTACTCC GATGACGACT GAGAGTTGTA AGATGAGGAG GTTTTTTCTT CTCTTTCCAT CTTCTGGGGT TCCTGAAAGG

Figure 14
(continued)

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5410 TTCAAGATTG CTAAGTTTTT 5420 5430 5440 5450 5460 5470 5480 5490
 AAGCTTAAAC GATTCAAAA ACTCAGTACG ACACAATCA TTATCTTGAG AACGAACGA AGGATAATG TGGTGTTC TTTTTCGACG
 5500 5510 5520 5530 5540 5550 5560 5570 5580
 ACTGCTATAC AAGAAATTA TGGAAAATA TTCTGTAAAC TTATAAATCAT AACATACTGT TTTTTCCTTAC
 TGACGATATG TTCTTTTAAT ACCTTTTAT AAGACATTGG AATATTTCAT CCGTATTGTC AATATTAGTA TTGTATGACA AAAAAGAAATG
 5590 5600 5610 5620 5630 5640 5650 5660 5670
 TCCACACAG CATAGAGTGT CTGCTATTAA TAACTATGCT CAAAATTTGT GTACCTTTAG CTTTTTAAT TTAAAGGGG TTATAAAGGA
 AGGTGTGTC GTATCTACA GACGATAATT ATTGATACGA GTTTTAAACA CATGGAATC GAAAATTTAA ACATTTCCCC AATTTATCCT
 5680 5690 5700 5710 5720 5730 5740 5750 5760
 ATATTGATG TATAGTCCCT TGACTAGAGA TCATAATCAG CCATACCACA TTGTAGAGG TTTTACTTGC TTAAATAAAC CTCCCACACC
 TATNAACTAC ATATCACGGA ACTGATCTCT AGTATTAGTC GGTATGGTGT AAACATCTCC AAAATGAAACG AATTTTTTG GAGGTGTGG
 5770 5780 5790 5800 5810 5820 5830 5840 5850
 TCCCTCGAA CCTGAACAT AAATGAATG CAATTGTTGT TGTAACTTG TTTATTCAG CTTATAATGG TTACAAATAA AGCAATAGCA
 AGGGGACTT GGACTTTGTA TTTTACTTAC GTTAAACACA ACAATTGAAC AATAACGTC GAATATTACC AATGTTTAT TCGTTATCGT
 5860 5870 5880 5890 5900 5910 5920 5930 5940
 TCACAAATTT CACAAATANA GCATTTTATT CACTGCATTC TAGTGTGGT TTGTCCAAC TCATCAATGT ATCTTATCAT GTCTGGATCG
 AGTGTATAA GTGTTTATTT CGTAAAAAAA GTGACGTAG ATCAACACCA AACAGGTTG AGTAGTTACA TAGAATAGTA CAGACCTAGC
 5950 5960 5970 5980 5990 6000 6010 6020 6030
 GCTGGATGAT CTTCCAGCG GGGATCTCA TGTGGGAGTT CTTGCCCCAC CCCAATCTGT TTATTGACAG TTATAATGGT TACAAATATA
 CGACCTACTA GGAGGTGCG CCCCTAGAGT ACGACCTCAA GAAGCGGTG GGGTTGAACA AATAACGTCG AATATTACCA ATGTTTATTT
 6040 6050 6060 6070 6080 6090 6100 6110 6120
 GCAATAGCAT CACAAATTC ACAATAAAG CATTTTTC ACTGCATCT ACTGCTGGT TGTCCAACAT CATCAATGTA TCTTATCATG
 CGTTATCGTA GTGTTAAAG TGTATTTC GTAAAAAAG TGACGTAGA TCACACCA ACAGGTTGA GTAGTTACAT AGAATAGTAC
 6130 6140 6150 6160 6170 6180 6190 6200 6210
 TCTGTATACC GTCGACCTCT AGCTAGAGCT TGGGTAATC ATGCTCATAG CTGTTTCTG TGTGAAATG TTATCCGCTC ACAATTCCAC
 AGACATATGG CAGCTGGAGA TCGATCTCGA ACCGCATTAG ACCGATATC TACCAGTATC GACNAAGGAC ACACTTTAAAC AATAGGCGAG TGTAAAGGTG
 6220 6230 6240 6250 6260 6270 6280 6290 6300
 ACAACATACG AGCCGAGC ATAAAGTGA AAGCTGGGG TGCTTAATGA GTGAGTAAC TCACATTAAAT TCGGTTGCGC TCACCTGCCG
 TGTGTATGC TCGGCTTCG TATTTACAT TTTGACCCCT ACGGATTACT CACTGATG AGTGTAAATTA ACGCAACCG AGTGACGGG

Figure 14
(continued)

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6310 6320 6330 6340 6350 6360 6370 6380 6390
 CTTTCAGTC GGGAAACCTG TCGTGCCAGC TGCATTAAATG AATCGGCCAA CGCGCGGGGA GAGCGGTTT GCGTATTGGG CGCTCTTTCCG
 GAAAGTTCAG CCTTTGGAC AGCAGGTTCG ACGTAATTAC TTAGCCGGTT GCGCGCCCTT CTCGCCCAA CGCATAACCC GCGAGAAGGC
 6400 6410 6420 6430 6440 6450 6460 6470 6480
 CTTCTCGCT CACTGACTCG CTGCGCTCGG TCGTTGCGCT GCGCGGAGCG GTATCAGCTC ACTCAAAGGC GGTAAATACG TTATCCACAG
 GAAGGACGA GTGACTGAGC GACGCGAGCC AGCAAGCCGA CCGCGCTCGC CATAGTCGAG TGAGTTTCCG CCAATTATGCC ANTAGGTGTC
 6490 6500 6510 6520 6530 6540 6550 6560 6570
 AATCAGGGA TAACGCAGGA AAGAATATGT GACCAAAAGG CCAGCAAAAG GCGAGGAACC GTAAAAAGGC CGCGTTGCTG CGGTTTTTCC
 TTAGTCCCTT ATTGCGTCTT TTCTTGATCA CTGTTTTTCC CGTCTGTTTC CGTCTCTTGG CATTTTTCG GCGCAACGAC CGCAAAAAGG
 6580 6590 6600 6610 6620 6630 6640 6650 6660
 ATAGGCTCCG CCCCCCTGAC GAGCATCACA AANAATCAGC CTCAAGTCAG AGGTGGCGAA ACCGACAGG ACTATAAGA TACCAGGCGT
 TATCCGAGGC GGGGGACTG CTGCTAGTGT TTTTAGCTGC GAGTTCAGTC TCCACCGCTT TGGGCTGTCC TGATATTTCT ATGGTCCGCA
 6670 6680 6690 6700 6710 6720 6730 6740 6750
 TTCCCTCTGG AAGCTCCCTC CTGCTCCGCTC CTGCTCCGCTT ACCGATACC TGTCGCGCTT TGTCGCGCTT TCTCCCTTCC GGNAGCGTGG
 AAGGGGACC TTGAGGGAG CAGCGGAGAG GACAAGGCTG GAGCGCGGAA TGGCTATAG ACAGCGGAA AGAGGGAAGC CCTTCGCACC
 6760 6770 6780 6790 6800 6810 6820 6830 6840
 CGCTTCTCA ATGCTCAGC TGTAGGTATC TCAGTTGCGT GTAGGTCGTT CGCTCCAGC TGGGCTGTGT GCACGAACCC CCGGTTTCAGC
 GCGAAAGAT TACGAGTCCG ACATCCATAG AGTCAAGCCA CATCCAGCAA CATCCAGCAA CCGAGGTTCC ACCCACACA CGTGTCTGG GGGCAAGTCCG
 6850 6860 6870 6880 6890 6900 6910 6920 6930
 CCGACCGCTG CGCCTTATCC GGTAACTATC GTCTTGAGTC CNAACCGTA AGACAGGACT TATCCCACT ATATCCCACT ACTGTAACA
 GCGTGGCAG CCGGAATAG CCAATTGATAG CCAATTGATAG CAGAACTCAG GTTGGGCCAT GTTGGGCCAT TCTGTCTGA ATAGCGTGA CCGTCTCTCG TGACCATTTGT
 6940 6950 6960 6970 6980 6990 7000 7010 7020
 GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT CTTGAAGTGG TGGCTTAAT ACGGTACAC TAGAAGGACA GTATTGTTGTA
 CCTAATCTGC TCCTCCATA CATCCGCCAC GATCTCTCA GAACTTCACC ACCGATTTGA TCGCGATGT ATCTCTCTGT CATAAACCAT
 7030 7040 7050 7060 7070 7080 7090 7100 7110
 TCTGCGCTCT GCTGAAGCA GTTACCTTCG GAAAGAGT TGGTAGCTCT TGATCCGCA AACAAACCAAC CCGTGTGAGC GGTGTTTTT
 AGACGCGAGA CGACTTCCGT CAATGGAAGC CTTTTTCTCA ACCATCGAGA ACTAGGCCGT TTGTTTGGTG GCGACCATCG CCACCAAAA
 7120 7130 7140 7150 7160 7170 7180 7190 7200
 TTGTTTGCAA GCAGCAGATT ACCGCGCAGAA AAAAAGGATC TCAAGAGAT CCTTTGATCT TTTCTACGGG GTCTGAGCT CAGTGGAAAG
 AACAAAGTT CGTCTCTAA TGGCGGTCTT TTTTCTCTAG AGTTCTCTTA GGAACCTAGA AAAGATGCCC CAGACTGGA GTCACCTTGC

Figure 14
(continued)

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7210	7220	7230	7240	7250	7260	7270	7280	7290
AAACTCAG	TTAAGGAT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	ACCTAGATCC	TTTTAAATTA	AAATGAAGT	TTTAAATCA
TTTTCAGTC	AATTCCTAA	AACCACTACT	CTAATAGTTT	TTCTTAGAAG	TGGATCTAGG	AAAATTTAAT	TTTTACTTCA	AAATTTAGTT
7300	7310	7320	7330	7340	7350	7360	7370	7380
TCATAAGTAT	ATATGAGTAA	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTTCA
AGATTTCATA	TATACTCAAT	TGAACCCAGAC	TGTCAATGGT	TACGAATTAG	TCACTCCGTG	GATAGAGTCG	CTAGACAGAT	AAAGCAAGTA
7390	7400	7410	7420	7430	7440	7450	7460	7470
CCATAGTTCC	CTGACTCCCC	GTCGTGTAGA	TAACTACGAT	ACGGGAGGCG	TTACCATCTG	GCCCCAGTGC	TGCAATGATA	CCGGAGAGCC
GGTATCAACG	GACTGAGGGG	CAGCACATCT	ATTGATGCTA	TGCCCCCTCCG	AATGGTAGAC	CGGGGTCACG	ACGTTACTAT	GGCGCTCTGG
7480	7490	7500	7510	7520	7530	7540	7550	7560
CAGGCTCACC	GGCTCCAGAT	TTATCAGCAA	TAAACGAGCC	AGCCGGAGGG	GGCGAGCGCA	GAAGTGTGCC	TGCAACTTTA	TCCGCTTCCA
GTCCGAGTGG	CCGAGGTCTA	AATAGTCGTT	ATTGGTCCG	TGGGCTTCC	CGGCTCGCGT	CTTCACCCAGG	ACGTTGAAAT	AGCGGAGGT
7570	7580	7590	7600	7610	7620	7630	7640	7650
TCAGATCTAT	TAATGTTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCCGCCAGT	AATAGTTTGC	GCAAGTTTGT	TGCCATTGCT	ACAGGCATCG
AGGTCAAGATA	ATTAACAACG	GGCCTTCGAT	CTCATTCATC	AAGCGTCAA	TTATCAAAACG	CGTTGCAACA	ACGGTAACGA	TGTCCTGAGC
7660	7670	7680	7690	7700	7710	7720	7730	7740
TGGTGTCAGG	CTCCTTCGGT	GGTATGCGTT	CATTACAGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTGTGCNAAA
ACCACAGTCC	GACGAGCCAA	CCATACCGAA	GTAAGTCGAG	GCCAAGGGTT	GCTAGTTCCG	CTCAATGTAC	TAGGGGGTAC	AACAGGTTTT
7750	7760	7770	7780	7790	7800	7810	7820	7830
AAGCGGTTAG	CTCCTTCGGT	CTTCGGATCG	TTGTCAAGAG	TAAGTTGGCC	GCAGTGTAT	CACATCATGT	TATGGCAGCA	CTGCATTAAT
TTCCGCCAATC	GAGGAAGCCA	GGAGGCTAGC	AACAGTCTTC	ATTCNAACCG	CGTCACNATA	GTGAGTACCA	ATACCGTCTG	GACGTATTAA
7840	7850	7860	7870	7880	7890	7900	7910	7920
CTCTTACTGT	CATGCCATCC	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGGGACCGA
GAGAAATGACA	GTACGGTAGG	CATTCTACGA	AAAGACACTG	ACCACCTATG	AGTTGGTTCA	GTAAGACTCT	TATCACATAC	GCGCTGGCT
7930	7940	7950	7960	7970	7980	7990	8000	8010
GTTCCTCTTG	CCCGGCGTCA	ATACGGGATA	ATACGGCGCC	ACATAGCAGA	ACTTTAAAG	TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC
CAACGAGAAC	GGGCGGAGT	TATGCCCTAT	TATGGCGCGG	TGTATCTCTCT	TGAAATTTTC	ACGAGTAGTA	ACCTTTTGCA	AGAAGCCCCG
8020	8030	8040	8050	8060	8070	8080	8090	8100
GAAACTCTC	AAGGATCTTA	CCGCTGTGTA	GATCCAGTTC	GATGTAAACC	ACTCGTGACAC	CCAACTGATC	TTTACAGCATCT	TTTACTTTCA
CTTTTGAGAG	TTCTTAGAAT	GGGACAACT	CTAGTCAAG	CTACATTTGGG	TGAGCACGTG	GGTTGACTAG	AAGTCTGTAGA	AAATGAAAGT

Figure 14
(continued)

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8110      8120      8130      8140      8150      8160      8170      8180      8190
CCAGCGTTTC TGGGTGAGCA AATACAGGAA GGCAAAATGC CGCAAAAG GGNATAGGG CGACACGGA ATGTTGAATA CTCATACTCT
GGTCGCAAG ACCACTCGT TTTGTCCCTT CCGTTTACG GCGTTTTC CTTATTCCTT TACAACCTTAT GAGTATGAGA

8200      8210      8220      8230      8240      8250      8260      8270      8280
TCCTTTTTC AATATTGGA AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT TGAATGTAT TTAGAAAAT AAACAATAG
AGGAANAAGT TATAATTAAT TCGTAATAG TCCCAATAAC AGAGTACTCG CCTATGTATA AACTTACATA AATCTTTTAA TTGTTTATC

8290      8300      8310      8320      8330
GGGTCCGGG CACATTTCCC CGAAAGTGC CACCTGAGT C
CCCAAGGCG GTGTAAAGG GCTTTTCACG GTGGACTGCA G
```

Figure 14
(continued)

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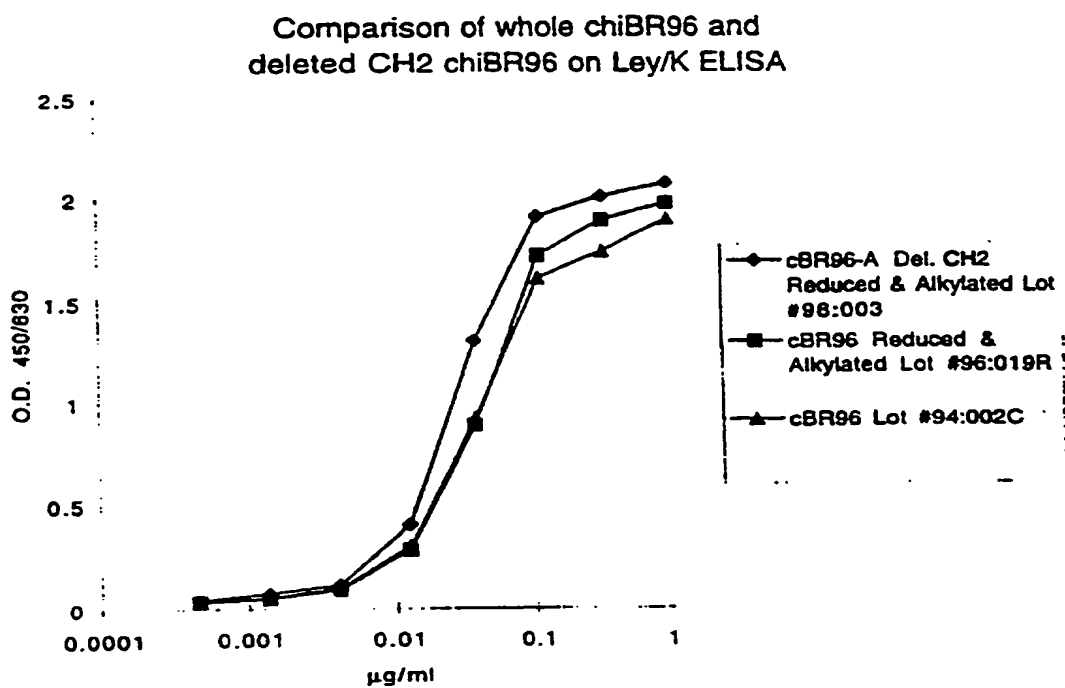


Figure 15

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hBR96-2B: L235 to A235 and G237 to A237

hBR96-2C: E318 to S318, K320 to S320, and K322 to S322

hBR96-2D: P331 to A331

hBR96-2E: L235 to A235, G237 to A237, E318 to S318, K320 to S320, and K322 to S322

hBR96-2F: L235 to A235, G237 to A237, and P331 to A331

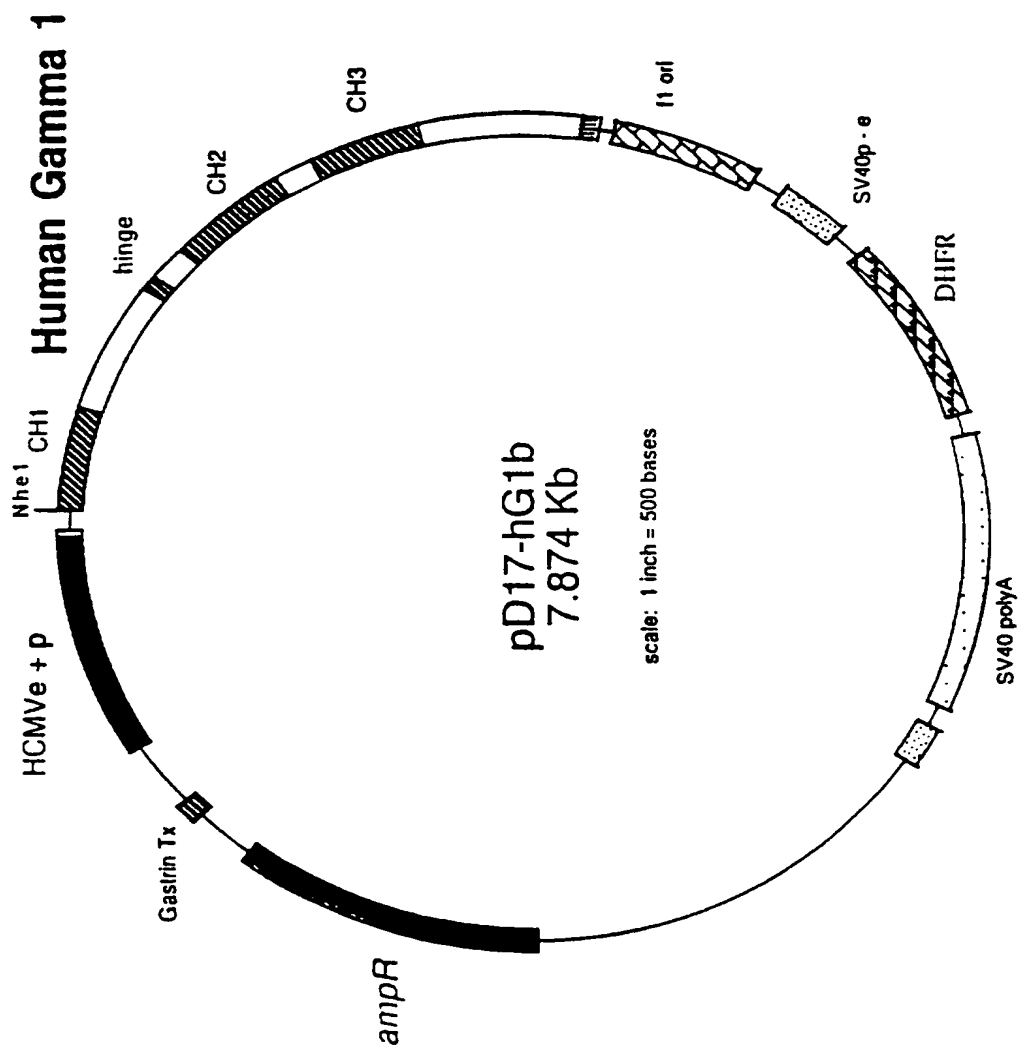
hBR96-2G: E318 to S318, K320 to S320, K322 to S322, and P331 to A331

hBR96-2H: L235 to A235, G237 to A237, E318 to S318, K320 to S320, K322 to S322, and P331 to A331

Figure 16

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FIGURE 17



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FIGURE 18A

1 GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC
51 GGTCAATCGA TTGGAATTCT TCGGCGCCGT TGCTAGCCAC CATGGAGTTG
101 TGGTTAAGCT TGGTCTTCCT TGTCTTGTT TTAAAAGGTG TCCAGTGTGA
151 AGTGCAACTG GTGGAGTCTG GGGGAGGCTT AGTGCAGCCT GGAGGGTCCC
201 TCGGACTTTC CTGTGCTGCA TCTGGATTCC CGTTCAGTGA CTATTACATG
251 TATTGGGTTC GCCAGGCTCC AGGCAAGGGA CTGGAGTGGG TCTCATACAT
301 TAGTCAAGAT GGTGATATAA CCGACTATGC AGACTCCGTA AAGGGTCGAT
351 TCACCATCTC CAGAGACAAT GCAAAGAACA GCCTGTACCT GCAAATGAAC
401 AGCCTGAGGG ACGAGGACAC AGCCGTGTAT TACTGTGCAA GAGGCCTGGC
451 GGACGGGGCC TGGTTTGCTT ACTGGGGCCA AGGGACTCTG GTCACGGTCT
501 CTTCCGCTAG CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC
551 AAGAGCACCT CTGGGGGCAC AGCGGCCCTG GGCTGCCTGG TCAAGGACTA
601 CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG
651 GCGTGACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT CTACTCCCTC
701 AGCAGCGTGG TCACCGTGCC CTCCAGCAGC TTGGGCACCC AGACCTACAT
751 CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTGGAC AAGAAAGTTG
801 GTGAGAGGCC AGCACAGGGA GGGAGGGTGT CTGCTGGAAG CCAGGCTCAG
851 CGCTCCTGCC TGGACGCATC CCGGCTATGC AGCCCCAGTC CAGGGCAGCA
901 AGGCAGGCCC CGTCTGCCTC TTCACCCGGA GGCCTCTGCC CGCCCCACTC
951 ATGCTCAGGG AGAGGGTCTT CTGGCTTTT CCCCAGGCTC TGGGCAGGCA
1001 CAGGCTAGGT GCCCCTAACC CAGGCCCTGC ACACAAAGGG GCAGGTGCTG
1051 GGCTCAGACC TGCCAAGAGC CATATCCGGG AGGACCCTGC CCCTGACCTA
1101 AGCCCACCCC AAAGGCCAAA CTCTCCACTC CCTCAGCTCG GACACCTTCT
1151 CTCCTCCCAG ATTCCAGTAA CTCCCAATCT TCTCTTGCA GAGCCCAAAT
1201 CTTGTGACAA AACTCACACA TGCCCACCGT GCCCAGGTAA GCCAGCCCAG
1251 GCCTCGCCCT CCAGCTCAAG GCGGGACAGG TGCCCTAGAG TAGCCTGCAT
1301 CCASGGACAG GCCCCAGCCC GGTGCTGACA CGTCCACCTC CATCTCTTCC

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1351 TCAGCACCTG AACTC²³⁵CTGGG²³⁷GGGACCGTCA GTCTTCCTCT TCCCCCAA
 1401 ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG
 1451 TGGTGGACCT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
 1501 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
 1551 CAACAGCAGC TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
 1601 GGCTGAATGG CAAG³¹⁸EAGTAC³²⁰ ~~AAGTGC~~³²²AGG TCTCCAACAA AGCCCTCCCA
 1651 G³³¹CC³³¹CCATCG AGAAAACCAT CTCCAAAGCC AAAGGTGGGA CCCGTGGGGT
 1701 GCGAGGGCCA CATGGACAGA GGCCGGCTCG GCCCACCCTC TGCCCTGAGA
 1751 GTGACCGCTG TACCAACCTC TGTCCCTACA GGGCAGCCCC GAGAACCACA
 1801 GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG AACCAGGTCA
 1851 GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
 1901 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT
 1951 GCTGGACTCC GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA
 2001 AGAGCAGGTG GCAGCAGGGG AACGTCTTCT CATGCTCCGT GATGCATGAG
 2051 GCTCTGCACA ACCACTACAC GCAGAAGAGC CTCTCCCTGT CTCCGGGTAA
 2101 ATGAGTGCGA CGGCCGGCAA GCCCCCGCTC CCCGGGCTCT CGCGGTCGCA
 2151 CGAGGATGCT TGGCACGTAC CCCCTGTACA TACTTCCCGG GCGCCCAGCA
 2201 TGGAAATAAA GCACCCAGCG CTGCCCTGGG CCCCTGCGAG ACTGTGATGG
 2251 TTCTTTCCAC GGGTCAGGCC GAGTCTGAGG CCTGAGTGGC ATGAGGGAGG
 2301 CAGAGCGGGT CCCACTGTCC CCACACTGGC CCAGGCTGTG CAGGTGTGCC
 2351 TGGGCCCCCT AGGGTGGGGC TCAGCCAGGG GCTGCCCTCG GCAGGGTGGG
 2401 GGATTTGCCA GCGTGGCCCT CCCTCCAGCA GCACCTGCCC TGGGCTGGGC
 2451 CACGGGAAGC CCTAGGAGCC CCTGGGGACA GACACACAGC CCCTGCCTCT
 2501 GTAGGAGACT GTCCTGTTCT GTGAGCGCCC CTGTCCTCCC GACCTCCATG
 2551 CCCACTCGGG GGCATGCCTA GTCCATGTGC GTAGGGACAG GCCCTCCCTC
 2601 ACCCATCTAC CCCCACGGCA CTAACCCCTG GCTGCCCTGC CCAGCCTCGC
 2651 ACCCGCATGG GGACACAACC GACTCCGGGG ACATGCACTC TCGGGCCCTG
 2701 TGGAGGGACT GGTGCAGATG CCCACACACA CACTCAGCCC AGACCCGTTC
 2751 AACAAACCCC GCACTGAGGT TGGCCGGCCA CACGGCCACC ACACACACAC
 2801 GTGCACGCCT CACACACGGA GCCTCACCCG GGCGAACTGC ACAGCACCCA

FIGURE 18B

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2851 GACCAGAGCA AGG CCTCGC ACACGTGAAC ACTCCTCGGA CACAGGCCCC
2901 CACGAGCCCC ACGCGGCACC TCAAGGCCCA CGAGCCTCTC GGCAGCTTCT
2951 CCACATGCTG ACCTGCTCAG ACAAACCCAG CCCTCCTCTC ACAAGGGTGC
3001 CCCTGCAGCC GCCACACACA CACAGGGGAT CACACACCAC GTCACGTCCC
3051 TGGCCCTGGC CCACTTCCCA GTGCCGCCCT TCCCTGCAGG ACGGATCAGC
3101 CTCGACTGTG CTTTCTAGTT GCCAGCCATC TGTGTGTTGC CCCTCCCCCG
3151 TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA
3201 AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT CTATTCTGGG
3251 GGGTGGGGTG GGGCAGGACA GCAAGGGGGA GGATTGGGAA GACAATAGCA
3301 GGCATGCTGG GGATGCGGTG GGCTCTATGG CTTCTGAGGC GGAAAGAACC
3351 AGCTGGGGCT CTAGGGGGTA TCCCCACGCG CCCTGTAGCG GCGCATTAAG
3401 CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG
3451 CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCTTTTCT CGCCACGTTC
3501 GCCGGGCCTC TCAAAAAAGG GAAAAAAGC ATGCATCTCA ATTAGTCAGC
3551 AACCATAGTC CCGCCCCTAA CTCGCCCAT CCCGCCCTA ACTCCGCCCA
3601 GTTCCGCCCC TTCTCCGCCC CATGGCTGAC TAATTTTTTT TATTTATGCA
3651 GAGGCCGAGG CCGCCTCGGC CTCTGAGCTA TTCCAGAAGT AGTGAGGAGG
3701 CTTTTTTTGA GGCCTAGGCT TTTGCAAAAA GCTTGGACAG CTCAGGGCTG
3751 CGATTTTCGG CCAAACTTGA CGGCAATCCT AGCGTGAAGG CTGGTAGGAT
3801 TTTATCCCCG CTGCCATCAT GGTTCGACCA TTGAACTGCA TCGTCGCCGT
3851 GTCCCAAAAT ATGGGGATTG GCAAGAACGG AGACCTACCC TGGCCTCCGC
3901 TCAGGAACGA GTTCAAGTAC TTCCAAAGAA TGACCACAAC CTCTTCAGTG
3951 GAAGGTAAAC AGAATCTGGT GATTATGGGT AGGAAAACCT GGTTCCTCCAT
4001 TCCTGAGAAG AATCGACCTT TAAAGGACAG AATTAATATA GTTCTCAGTA
4051 GAGAACTCAA AGAACCACCA CGAGGAGCTC ATTTTCTTGC CAAAAGTTTG
4101 GATGATGCCT TAAGACTTAT TGAACAACCG GAATTGGCAA GTAAAGTAGA
4151 CATGGTTTGG ATAGTCGGAG GCAGTTCTGT TTACCAGGAA GCCATGAATC
4201 AACCAGGCCA CCTTAGACTC TTTGTGACAA GGATCATGCA GGAATTTGAA
4251 AGTGACACGT TTTTCCCAGA AATTGATTTG GGGAAATATA AACTTCTCCC
4301 AGAATACCCA GGCCTCCTCT CTGAGGTCCA GGAGGAAAAA GGCATCAAGT

FIGURE 18C

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4351 ATAAGTTTGA AGTCTACGAG AAGAAAGACT AACAGGAAGA TGCTTTC AAG
4401 TTCTCTGCTC CCCTCCTAAA GCTATGCATT TTTATAAGAC CATGGGACTT
4451 TEGCTGGCTT TAGATCTCTT TGTGAAGGAA CCTTACTTCT GTGGTGTGAC
4501 ATAATTGGAC AAACCTACCTA CAGAGATTTA AAGCTCTAAG GTAAATATAA
4551 AATTTTTTAAG TGTATAATGT GTTAAACTAC TGATTCTAAT TGTTTGTGTA
4601 TTTTAGATTG CAACCTATGG AACTGATGAA TGGGAGCAGT GGTGGAATGC
4651 CTTTAATGAG GAAAACCTGT TTTGCTCAGA AGAAATGCCA TCTAGTGATG
4701 ATGAGGCTAC TGCTGACTCT CAACATTCTA CTCCTCCAAA AAAGAAGAGA
4751 AAGGTAGAAG ACCCCAAGGA CTTTCCTTCA GAATTGCTAA GTTTTTTGAG
4801 TCATGCTGTG TTTAGTAATA GAACTCTTGC TTGCTTTGCT ATTTACACCA
4851 CAAAGGAAAA AGCTGCACTG CTATACAAGA AAATTATGGA AAAATATTCT
4901 GTAACCTTTA TAAGTAGGCA TAACAGTTAT AATCATAACA TACTGTTTTT
4951 TCTTACTCCA CACAGGCATA GAGTGTCTGC TATTAATAAC TATGCTCAAA
5001 AATTGTGTAC CTTTAGCTTT TTAATTTGTA AAGGGGTAA TAAGGAATAT
5051 TTGATGTATA GTGCCCTGAC TAGAGATCAT AATCAGCCAT ACCACATTTG
5101 TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG
5151 AAACATAAAA TGAATGCAAT TGTGTGTGTT AACTTGTTTA TTGCAGCTTA
5201 TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTCACA AATAAAGCAT
5251 TTTTTTCACT GCATTCTAGT TGTGGTTTGT CCAAACCTCAT CAATGTATCT
5301 TATCATGTCT GGATCGGCTG GATGATCCTC CAGCGCGGGG ATCTCATGCT
5351 GGAGTTCTTC GCCCACCCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA
5401 AATAAAGCAA TAGCATCACA AATTTACAA ATAAAGCATT TTTTCACTG
5451 CATTCTAGTT GTGGTTTGTG CAAACTCATC AATGTATCTT ATCATGTCTG
5501 TATACCGTCG ACCTCTAGCT AGAGCTTGGC GTAATCATGG TCATAGCTGT
5551 TTCCTGTGTG AAATTGTTAT CCGCTCACAA TTCCACACAA CATACGAGCC
5601 GGAAGCATAA AGTGTAAGC CTGGGGTGCC TAATGAGTGA GCTAACTCAC
5651 ATTAATTGCG TTGCGCTCAC TGCCCGCTTT CCAGTCGGGA AACCTGTCGT
5701 GCCAGCTGCA TTAATGAATC GGCCAACCGG CGGGGAGAGG CGGTTTSCGT
5751 ATTGGGCGCT CTTCCGCTTC CTCGCTCACT GACTCGCTGC GCTCGGTCGT
5801 TCGGCTGCGG CGAGCGGTAT CAGCTCACTC AAAGGCGGTA ATACGGTTAT

FIGURE 18D

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5351 CCACAGAATC AGGGGATAAC GCAGGAAAGA ACATGTGAGC AAAAGGCCAG
5901 CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG
5951 GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT
6001 GGCAGAAACCC GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC
6051 TCCCTCGTGC GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC
6101 CGCCTTTCTC CCTTCGGGAA GCGTGGCGCT TTCTCAATGC TCACGCTGTA
6151 GGTATCTCAG TTCGGTGTAG GTCGTTGCT CCAAGCTGGG CTGTGTGCAC
6201 GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA ACTATCGTCT
6251 TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG
6301 GTAACAGGAT TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG
6351 AAGTGGTGGC CTAACCTACGG CTACACTAGA AGGACAGTAT TTGGTATCTG
6401 CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT
6451 CCGGCAAACA AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG
6501 CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC
6551 TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTAA GGGATTTTGG
6601 TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATTAAAAA
6651 TGAAGTTTTA AATCAATCTA AAGTATATAT GAGTAACTT GGTCTGACAG
6701 TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC TGTCTATTTT
6751 GTTCATCCAT AGTTGCCTGA CTCCCCGTCG TGTAGATAAC TACGATACGG
6801 GAGGGCTTAC CATCTGGCCC CAGTGCTGCA ATGATACCGC GAGACCCACG
6851 CTCACCGGCT CCAGATTTAT CAGCAATAAA CCAGCCAGCC GGAAGGGCCG
6901 AGCGCAGAAG TGGTCCTGCA ACTTTATCCG CCTCCATCCA GTCTATTAAT
6951 TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG CCAGTTAATA GTTTGCGCAA
7001 CGTTGTTGCC ATTGCTACAG GCATCGTGGT GTCACGCTCG TCGTTTGTA
7051 TGGCTTCATT CAGCTCCGGT TCCCAACGAT CAAGGCGAGT TACATGATCC
7101 CCCATGTTGT GCAAAAAAGC GGTTAGCTCC TTCGGTCCTC CGATCGTTGT
7151 CAGAAGTAAG TTGGCCGCAG TGTTATCACT CATGGTTATG GCAGCACTGC
7201 ATAATTCTCT TACTGTCATG CCATCCGTAA GATGCTTTTC TGTGACTGGT
7251 GAGTACTCAA CCAAGTCATT CTGAGAATAG TGTATGCGGC GACCGAGTTG
7301 CTCTTGCCCC GCSTCAATAC GGGATAATAC CGCGCCACAT AGCAGAACTT

FIGURE 18E

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7351 TAAAAGTGCT CATCATTGGA AAACGTTCTT CGGGGCGAAA ACTCTCAAGG
7401 ATCTTACCGC TGTGAGATC CAGTTCGATG TAACCCACTC GTGCACCCAA
7451 CTGATCTTCA GCATCTTTTA CTTTCACCAG CGTTTCTGGG TGAGCAAAAA
7501 CAGGAAGGCA AAATGCCGCA AAAAAGGGAA TAAGGGCGAC ACGGAAATGT
7551 TGAATACTCA TACTCTTCCT TTTTCAATAT TATTGAAGCA TTTATCAGGG
7601 TTATTGTCTC ATGAGCGGAT ACATATTTGA ATGTATTTAG AAAAATAAAC
7651 AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCACC TGACGTCGAC
7701 GGATCGGGAG ATCTGCTAGG TGACCTGAGG CGCGCCGGCT TCGAATAGCC
7751 AGAGTAACCT TTTTTTTTAA TTTTATTTTA TTTTATTTTT GAGATGGAGT
7801 TTGGCGCCGA TCTCCCGATC CCCTATGGTC GACTCTCAGT ACAATCTGCT
7851 CTGATGCCGC ATAGTTAAGC CAGTATCTGC TCCCTGCTTG TGTGTTGGAG
7901 GTCGCTGAGT AGTGCGCGAG CAAAATTTAA GCTACAACAA GGCAAGGCTT
7951 GACCGACAAT TGCATGAAGA ATCTGCTTAG GGTTAGGCGT TTTGCGCTGC
8001 TTCGCGATGT ACGGGCCAGA TATACGCGTT GACATTGATT ATTGACTAGT
8051 TATTAATAGT AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA
8101 GTTCCGCGTT ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA
8151 ACGACCCCCG CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG
8201 CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGACTATT TACGGTAAAC
8251 TGCCCCACTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA
8301 TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG
8351 ACCTTATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC
8401 TATTACCATG GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC
8451 GGTTTGA CTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG
8501 AGTTTGT TTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA
8551 CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT
8601 ATATAAGCAG AGCTCTCTGG CTAAGTAGAG AACCCACTGC TTA CTGGCTT
8651 ATCGAAATTA ATACGACTCA CTATAGGGAG ACCCAAGCTT

FIGURE 18F

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FIGURE 19 A
pD17-hG1b

10 20 30 40 50 60
GGTACCAATT TAAATTGATA TCTCTTAGG TCTCGAGTCT CTAGATAACC GGTCAATCGA
CCATGGTTAA ATTTAACTAT AGAGGAATCC AGAGCTCAGA GATCTATTGG CCAGTTAGCT
70 80 90 100 110 120
TTGGAATICT TGCGGCGGCT TGCTAGCACC AAGGGCCCAT CCGTCTTCCC CCGGCACCC
AACCTTAAGA ACGCCGCGA ACGATCGTGG TTCCCGGGTA GCCAGAAGGG GGACCGTGGG
130 140 150 160 170 180
TCC'TCCAAGA GCACCTCTGG GGGCACAGCG GCCCTGGGCT GCCTGGTCAA GGACTACTTC
AGGAGGTICT CGTGGAGACC CCCGTGTCGC CCGGACCCCA CCGACCAGTT CCTGATGAAG
190 200 210 220 230 240
CCCGAACCGG TGACGGTGTG GTGGAACCTCA GCGGCCCTGA CCAGCGGCGT GCACACCTTC
GGCTTGGCC ACTGCCACAG CACCTTGAGT CCGCGGGGACT GGTGGCGCA CGTGTGGAAG
250 260 270 280 290 300
CCGGCIGTCC TACAGTCTTC AGGACTCTAC TCCCTCAGCA GCGTGGTCAC CGTGCCTCC
GGCCGACAGG ATGTCAGGAG TCCTGAGATG AGGAGTCGT CGCACCATG GCACGGGAGG
310 320 330 340 350 360
AGCAGCTGG GCACCCAGAC CTACATCTGC AACGTGATC ACAAGCCCGA CAACACCAAG
TCGTGCAACC CGTGGGTCTG GATGTAGACG TTGCACCTAG TGTTCGGGTC GTTGTGGTTC
370 380 390 400 410 420
G'TGGACNAGA AAGTTGGTGA GAGGCCAGCA CAGGGAGGGA GGGTGTCTGC TGGAAAGCCAG
CACCTGTCTT T'TCAACCACT C'TCCGGTCTGT GTCCCTCCCT CCCACAGACG ACCTTCGGTC
430 440 450 460 470 480
GCTCAGCGCT CCTGCCTGGA CGCATCCCGG CTA'IGCAGCC CCAGTCCAGG GCAGCAAGGC
CGAGTCGCGA GGACGGACCT GCGTAGGGCC GATACGTCGG GGTACAGGTCC CGTCGTTCGG
490 500 510 520 530 540
AGGCCCGTC TGCCCTCTTCA CCCGGAGGCC TCTGCCCGCC CCACCTCATGC TCAGGGAGAG
TCCGGGCGAG ACGGAGAAGT GGGCTCCCGG AGACGGGCGG GGTGAGTAGG AGTCCCTCTC
550 560 570 580 590 600
GGTCTCTGG CTTTTTCCC AGGCTCTGGG CAGGCACAGG CTAGGTGCC CTAACCCAGG
CCNAGNACC GAAAAGGGG TCCGAGACCC GTCCGTGTC GATCCACGGG GATTCGGTCC

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FIGURE 19B

pD17-hG1b

610 620 630 640 650 660
 CCCTGCACAC AAAGGGCAG GTGCTGGCT CAGACCTGCC AAGAGCCATA TCCGGGAGGA
 GGGACGTTG TTTCCCGCTC CACGACCCGA GTCTGGACGG TTCTCGGTAT AGGCCCTCCT

670 680 690 700 710 720
 CCCTGCCCT GACCTAAGCC CACCCCAAAG GCCAACTCT CCACTCCCTC AGCTCGGACA
 GGGACGGGA CTGGATTCTG GTGGGTTTC CGTTTGAGA GGTGAGGGAG TCGAGCCTGT

730 740 750 760 770 780
 CCTTCTCTC TCCAGATTTC CAGTAACCTC CAATCTTCTC TCTGCAGAGC CCAATCTTTG
 GGAAGAGAGG AGGGTCTAAG GTCATTTGAGG GTTAGAAGAG AGACGTCTCG GGTTTAGAAC

790 800 810 820 830 840
 TGACAAACT CACACATGCC CACCGTGCC AGTAAGCCA GCCCAGGCCCT CGCCCTCCAG
 ACTGTTTGA GTGTGTACGG GTGGCACGG TCCATTCTGGT CGGTCCGGA GCGGAGGTC

850 860 870 880 890 900
 CTCAGGCGG GACAGTGCC CTAGAGTAGC CTGCATCCAG GGACAGGCC CAGCCGGGTG
 GAGTTCCGCC CTGTCCACGG GATCTCATCG GACGTAGGTC CCTGTCCGG GTCCGGCCAC

910 920 930 940 950 960
 CTGACACGTC CACCTCCATC TCTTCTCAG CACCTGAAC TCTGGGGA CCGTCAGTCT
 GACTGTGCAG GTGGAGGTAG AGAAGGATC GTGGACTTGA GACCCCTT GGCAGTCAGA

970 980 990 1000 1010 1020
 TCTCTCTTCCC CCCAAACCC AAGGACACC TCATGATCTC CCGGACCCCT GAGGTCACAT
 AGGAGAGGG GGGTTTGGG TTCTCTGTGG AGTACITAGAG GCGCTGGGA CTCCAGTGT

1030 1040 1050 1060 1070 1080
 GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG TACGTGACG
 CGCACCAACA CCTGCACTCG GTGCTTCTGG GACTCCAGTT CAAGTTGACC ATGCACCTGC

1090 1100 1110 1120 1130 1140
 GCGTGGAGGT GCATTAATGCC AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCAGGTACC
 CGCACCTCCA CGTATTACGG TTCTGTCTCG GCGCCCTCTC CGTCATGTTG TCGTGCATGG

1150 1160 1170 1180 1190 1200
 GTGTGCTCAG CGTCTCACC GTCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAGT
 CACACCACTC GCAGGAGTGG CAGGACGTGG TCTGACCCA CTATACGTTT CTTCTGTTCA

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FIGURE 19C

pD17-hG1b

322 1210 1220 1230 1240 1250 1260
 CCAAGGTCTC CAACAAGCC CTCCCAGCC CCAATCGAGAA AACCATCTCC AAAGCCAAAG
 CATTCCAGAG GTTGTTCGG GAGGTCCGG GATGCTCTT TTGGTAGAGG TTTCGGTTTC
 1270 1280 1290 1300 1310 1320
 GTGGACCCG TGGGGTGCGA GGGCCACATG GACAGAGGCC GGCTCGGCC ACCCTCTGCC
 CACCCCTGGC ACCCCACGCT CCGGTGTAC CTGTCTCCG CCGAGCCGG TGGGAGACGG
 1330 1340 1350 1360 1370 1380
 CTGAGAGTGA CCGCTGTACC AACCTCTGTC CCTACAGGGC AGCCCCGAGA ACCACAGGTG
 GACTCTCACT GGGCACATGG TTGGAGACAG GGATGTCCCG TCGGGCTCT TGGTGTCCAC
 1390 1400 1410 1420 1430 1440
 TACACCCTCG CCCCATCCCG GGATGAGCTG ACCAAGAACC AGGTCAGCCT GACCTGCCTG
 ATGTGGGACG GGGGTAGGGC CCTACTCGAC TGGTCTTGG TCCAGTCGA CTGGACGGAC
 1450 1460 1470 1480 1490 1500
 GTCAAAGGT TCTATCCAG CGACATCGCC GTGGAGTGG AGAGCAATGG GCAGCCGGAG
 CAGTTTCCGA AGATAGGCTC GCTGTAGCG CACCTCACCC TCTCGTTACC CGTCGGCCTC
 1510 1520 1530 1540 1550 1560
 AACAACTACA AGACCAGCC TCCCGTCTG GACTCCGACG GCTCCTCTT CCTCTACAGC
 TTGTTGATGT TCTGTGCGG AGGCACGAC CTGAGGCTGC CGAGGAAGAA GGAGATGTCG
 1570 1580 1590 1600 1610 1620
 AAGCTCACCG TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTCTCATG CTCCGTGATG
 TTTCGAGTGGC ACCTGTTCTC GTCCACCGTC GTCCCTTGC AGAAGATAC GAGGCACCTAC
 1630 1640 1650 1660 1670 1680
 CATGAGGCTC TGCACAACCA CTACACGCGA AAGAGCTCT CCCTGTCTCC GGTAAATGA
 GTACTCCGAG ACGTGTGGT GATGTGCTC TTCTCGGAGA GGGACAGAGG CCCATTTACT
 1690 1700 1710 1720 1730 1740
 GTGCGACGGC CGGCAAGCCC CCGCTCCCG GGCTCTCGCG GTCCGACGAG GATGCTTGGC
 CACGCTGCCG GCCGAGGGG GCGAGAGCG CAGCGTCTC CTACGAACCG
 1750 1760 1770 1780 1790 1800
 ACGTACCCCT TGTACATACT TCCCGGGCG CCAGCATGGA AATAAGCAC CCAGCGCTGC
 TCCAATGGGG ACAATATGA AGGCCCCGCG GTTCGTACCT TTATTTCTG TGTCCGACG

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FIGURE 19D

pD17-hG1b

1810	1820	1830	1840	1850	1860
CGTGGGCCC	TGGAGAC'IG	TGATGGT'CT	TTCCACGGGT	CAGGCCGAGT	CTGAGGCCCTG
GGACCCGGGG	ACGCT'IGAC	ACTACCAAGA	AAGGTGCCCA	GTCCGGCTCA	GACTCCGGAC
1870	1880	1890	1900	1910	1920
AGTGGCA'IGA	GGGAGGCAGA	GCGGGTCCCA	CTGTCCCCAC	ACTGGCCACG	GCTGTGCAGG
TCACCGTACT	CCCTCCGTCT	CGCCCAAGGT	GACAGGGGTG	TGACCCGGGTG	CGACACGTCC
1930	1940	1950	1960	1970	1980
TG'TGCC'TGGG	CCCCCTAGGG	TGGGGCTCAG	CCAGGGGGCTG	CCC'ICGGCAG	GGTGGGGGAT
ACACGGACCC	GGGGGATCCC	ACCCCGAGTC	GGTCCCCGAC	GGGAGCCGTC	CCACCCCCCTA
1990	2000	2010	2020	2030	2040
TTGCCAGCGT	GGCCCTCCCT	CCAGCAGCAC	CTGCCCTGGG	CTGGGCCACG	GGAAGCCCCTA
AACGGTCGCA	CCGGGAGGGA	GGTCGTCTGT	GACGGGACCC	GACCCGGTGC	CCTTCGGGAT
2050	2060	2070	2080	2090	2100
GGAGCCCC'TG	GGGACAGACA	CACAGCCCCCT	GCCTCTG'PAG	GAGACTGTCC	TGTTCTGTGA
CC'TCGGGAC	CCCTGTCTGT	G'GTCTGGGGA	CGGAGACATC	CTCTGACAGG	ACAAGACACT
2110	2120	2130	2140	2150	2160
GCGCCCC'TGT	CCTCCCGACC	TCCATGCCCA	CTCGGGGGCA	TGCTGGGGAT	GCGGTGGGCT
CGCGGGGACA	GGAGGGCTGG	AGGTACGGGT	GAGCCCCCGT	ACGACCCCTA	CGCCACCCGA
2170	2180	2190	2200	2210	2220
C'TA'IGGCT'ITC	TCAGGCGGAA	AGAACCAGCT	GGGGCTC'PAG	GGGGTATCCC	CACGCGCCCT
GATACCGNAG	ACTCCGCCCTT	TC'TTGGTCGA	CCCCGAGATC	CCCCATAGGG	GTGCGCGGGA
2230	2240	2250	2260	2270	2280
GTAGCGGGCG	ATTAAAGCGG	GCGGG'GTGG	TGGTTPACG'G	CAGCGTGACC	GCTACACTTG
CATCGCCGG	T'AATTCCGGC	CGCCCAACCC	ACCAATGCGC	GTCGCACTGG	CGATGTGAAC
2290	2300	2310	2320	2330	2340
CCAGCGCCCT	AGCGCCCGCT	CC'TTTCGGCTT	TC'TTCCCTTC	CTTTCCTGCC	ACGTTCCGCC
GG'TCGCGGA	TCGCGGGCGA	GGAAAGCGAA	AGAAGGGGAG	GAAAGAGCGG	TGCAAGCGGC
2350	2360	2370	2380	2390	2400
GCTTTCCTCCG	TCAAGCTCTA	AATCGGGGCA	TCCCTTTTAGG	GTTCCGATT	AGTGTCTTAC
CGAAGGGGC	AGTTCGAGAT	TTAGCCCCGT	AGGGAATCC	CAAGGCTAAA	TCACGNAATG

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FIGURE 19E

pD17-hG1b

2410 GGCACCTCGA CCCCCAAAA CTGTGATTAGG GTGATGGTTC ACGTAGTGGG CCATCGCCCT 2460
 CCGTGGAGCT GGGGTTTTTT GAACTAAATCC CACTACCAAG TGCATCACCC GGTAGCGGGA
 2470 GATAGACGGT TTTTCGCCCT TTGACGTGGG AGTCCACGTT CTTTAATAGT GGACTCTTGT 2520
 CTATCTGCCA AAAAGCGGGA AACTGCAACC TCAGGTGCAA GAAATTATCA CCTGAGAACA
 2530 TCCAAAC'TGG AACAACATC AACCCATATCT CGGTCTATTC TTTTGATTTA TAAGGGATT 2580
 AGGTTTGACC TTGTGTGAG TTGGGATAGA GCCAGATAAG AAAACTAAAT ATTCTCTTAA
 2590 TGGGGATTTC GGCCTATTGG TTAATAAATG AGCTGATTTA ACAAAAATTT AACGGGAATT 2640
 ACCCCATAAG CCGGATAACC AATTTTATTAC TCGACTAAAT TGTTTTAA TCGCGCTTAA
 2650 AATTCGTGG AATGTGTGTC AGTTAGGGTG TGGAAAGTCC CCAGGCTCCC CAGGCAGGCA 2700
 TTAAGACACC TTACACACAG TCAATCCCAC ACCTTTCAGG GGTCGAGGG GTCCGTCCGT
 2710 GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAT AGTCCCGCCC CTAAC'TCCGC 2760
 CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTA TCAGGGCGGG GATTGAGCG
 2770 CCATCCCGC CCTAATCCG CCCAGTTCCG CCCATTCTCC GCGGGTACCG ACTGATTAAA 2820
 GGTAAGGCGG GGATTGAGGC GGGTCAAGGC GGGTAAGAGG CCGGGTACCG ACTGATTAAA
 2830 TTTTATTATA TGCAGAGGCC GAGGCGGCGT CGGCCCTCTGA GCTATTCCAG AAGTAGTGAG 2880
 AAAAATAAAT ACGTCTCCG CTCCGGGCGA GCGGAGACT CGATAAGGTC TTCAATCACTC
 2890 GAGGCTTTT JGGAGGCCTA GGC'TTTTGCA AAAAGCTTGG ACAGCTCAGG GCTGCGATT 2940
 CTCCGAAAAA ACCTCCGGAT CCGAANAACGT TTTTCGAACC TGTGAGTCC CGACGCTAAA
 2950 CCGGCCAAAC TTGACGGCAA TCCTAGCGTG AAGGTGGTA GGATTTTATC CCGCTGCCA 3000
 GCGCGTTTTC AACTGCCGT AGGATCGCAC TTCCGACCAT CC'IAAAA'TAG GGGCGACGGT

FIGURE 19F

pD17-hG1b

3010 TCATGGTTCG ACCATTGAAC TGCATCGTCG CCGTGTCCTCA AAATATGGGG ATTGGCAAGA 3060
 AGTACCAAGC TGGTAACCTG ACGTAGCAGC GGCACAGGGT TTTATACCCC TAACCGTTCT
 3070 ACGGAGACCT ACCCTGGCCT CCGCTCAGGA ACGAGTTCAA GTACTTCCAA AGAATGACCA 3120
 TGCCCTCTGA TGGGACCGGA GCGAGTCCT TGCTCAAGTT CATGAAGGTT TCTTACTGGT
 3130 CAACCTCTTC AGTGAAGGT AAACAGAATC TGGTGATTTAT GGGTAGGAAA ACCTGGTCTT 3180
 GTTGGAGAAG TCACCTTCCA TTTGTCTTAG ACCACTAATA CCCATCTTTT TGGACCAAGA
 3190 CCATTCCTGA GAAGAATCGA CCTTTAAAGG ACAGAAATTAA TATAGTTCTC AGTAGAGAAC 3240
 GGTAAAGGACT CTTCTTAGCT GGAATTTTCC TGCTTTAATT ATATCAAGAG TCATCTCTTG
 3250 TCAGGAAC ACCACGAGGA GCTCATTTTC TTGCCAAAAG TTTGGATGAT GCCTTAAAGAC 3300
 AGTTTCTTGG TGGTGCTCCT CGAGTAAAG AACGGTTTTC AAACCTACTA CGGAATTTCTG
 3310 TTATTGAACA ACCGGAATTG GCAAGTAAAG TAGACATGGT TTGGATAGTC GGAGGCAGTT 3360
 AATAACTTGT TGGCCTTAAC CGTTCATTTT ATCTGTACCA AACCTATCAG CCTCCGTCAA
 3370 CTGTTTACCA GGAAGCCAAG AATCAACCAG GCCACCTTAG ACTCTTTGTG ACAAGGATCA 3420
 GACAAAATGGT CCTTCGGTAC TTAGTTGGTC CCGTGGAAIC TGAGAAACAC TGTTCTCTAGT
 3430 TGCAGGAATT TGAAGTGAC ACGTTTTC CAGAAATTGA TTTGGGAAA TATAAATTC 3480
 ACGTCTCTAA ACTTTTCACTG TGCAAAAAGG GTCTTTAACT AAACCCCTTT ATATTGAAG
 3490 TCCCAGAATA CCCAGGGCTC CTCTCTGAGG TCCAGGAGGA AAAAGGCATC AAGTATAAGT 3540
 AGGGTCATTAT GGGTCCGCCAG GAGAGACTCC AGGTCCACCT TTTTCCGTAG TTATATATCA
 3550 TTGAAGTCTA CGAGAAGAAA GACTAACAGG AAGATGCTTT CAAGTCTCTT GCTCCCCCTCC 3600
 NACTTCAGNT GCTCTTCTTT CTGATTGTCC TTCTACGAAA GTTCAAGAGA CGAGGGGAGG

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FIGURE 19C

pD17-hG1b

3610	3620	3630	3640	3650	3660
TAAAGCTATG	CATTTTATA	AGACCATGGG	ACTTTTGCTG	GCATTAGATC	TCTTTGTGAA
ATTTCGATAC	GTAATAATAT	TCTGGTACCC	TGAAAACGAC	CGAAATCTAG	AGAAACACTT
3670	3680	3690	3700	3710	3720
GGAACTTAC	TTCTGTGGTG	TGACATAAAT	GGACAAACTA	CCTACAGAGA	TTTAAAGCTC
CCTTGGAATG	AAGACACCCAC	ACTGTATTAA	CCTGTTTGAT	GGATGTCTCT	AAATTTTCGAG
3730	3740	3750	3760	3770	3780
TAAGGTAAAT	ATAAAATTTT	TAAGTGTATA	ATGTTGTAAA	CTACTGATTC	TAATTTGTTG
ATTTCANTTA	TATTTTAAAA	ATTACACATAT	TACACAATTT	GATGACTAAG	ATTAAACAAAC
3790	3800	3810	3820	3830	3840
TGTATTTTAG	ATTCCAACTT	ATGGAACCTG	TGAATGGGAG	CAGTGGTGA	ATGCCCTTTAA
ACATAAAATC	TAAGGTTGGA	TACCTTTGACT	ACTTACCCCTC	GTCACCCACCT	TACGGAAATT
3850	3860	3870	3880	3890	3900
TGAGGAAAC	CTGTTTTCCT	CAGAAAGAAAT	GCCATCTAGT	GATGATGAGG	CTACTGCTGA
ACTCCTTTTG	GACAAAACGA	GTCCTTCTTTA	CGGTAGATCA	CTACTACTCC	GATGACGACT
3910	3920	3930	3940	3950	3960
CTCTCAACAT	TCTACTCCTC	CAAAAAGAA	GAGAAAGGTA	GAAGACCCCA	AGGACTTTCC
GAGAGTTGTA	AGATGAGGAG	GTTTTTTTCTT	CTCTTTCCAT	CTTCTGGGGT	TCCTGAAAGG
3970	3980	3990	4000	4010	4020
TTTCAGANITG	CTAAGTTTTT	TGAGTCAATG	TGTGTTTAGT	AATAGAACTC	TTGCTTTCCT
AAGTCTTAAC	GATTCAAAAA	ACTCAGTACG	ACACAAATCA	TTATCTTGAG	AACGAACGAA
4030	4040	4050	4060	4070	4080
TTCTATTATAC	ACCACAAAGG	AAAAAGCTGC	ACTGCTATAC	AAGAAAAATTA	TGGAAAAATA
ACGATAAATG	TGGTGTTCCT	TTTTTTCGACG	TGACGATATG	TTCTTTTAAT	ACCTTTTTAT
4090	4100	4110	4120	4130	4140
TTCTGTAAAC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	TTTTTCTTAC
AAGACATTTGG	AAATATTTCAT	CCGTATTGTC	AATATTAGTA	TTGTATGACA	AAAAAGAATG
4150	4160	4170	4180	4190	4200
TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACTATGCT	CAAAAAATGT	GTACCTTTAG
AGGTGTGTCC	GTATCTCACA	GACGATAATT	ATTGATACGA	GTTTTTAACA	CATGGAAATC

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FIGURE 19H

pD17-hG1b

4210	4220	4230	4240	4250	4260
CTTTTAAAT	TGTAAAGGG	TAAATAAGGA	ATATTGATG	TATAGTGCCT	TGACTAGAGA
GAAAAATAA	ACATTTCCT	AAATATTCCT	TATAAACTAC	ATATCACGGA	ACTGATCTCT
4270	4280	4290	4300	4310	4320
TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTTACTTGC	TTTAAAAAAC	CTCCACACCC
AGTATTAGTC	GGTATGGTGT	AAACATCTCC	AAAATGAACG	AAATTTTGTG	GAGGGTGTGG
4330	4340	4350	4360	4370	4380
TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTGTG	TGTTAACITG	TTTTATTGCAG
AGGGGGACTT	GGACTTTGTA	TTTTACTTAC	GTTAACAAACA	ACAATTGAAC	AAATAACGTC
4390	4400	4410	4420	4430	4440
CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	GCATTTTTTTT
GAATATTACC	AATGTTTTAT	TCGTTATCGT	AGTGTTTTAA	GTGTTTATTT	CGTAAAAAAA
4450	4460	4470	4480	4490	4500
CACTGCATTC	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	GTCTGGATCG
GTGACGTAAG	ATCAACACCA	AACAGGTTTG	AGTAGTTTACA	TAGAATAGTA	CAGACCTAGC
4510	4520	4530	4540	4550	4560
GCTGGATGAT	CTCCAGGCG	GGGATCTCA	TGCTGGAGTT	CTTCGCCCCAC	CCCAACTTGT
CGACCTACTA	GGAGGTCGG	CCCCTAGAGT	ACGACCTCAA	GAAGCGGGTG	GGGTTGAACA
4570	4580	4590	4600	4610	4620
TTATTGTCAG	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTT	ACAAATAAAG
AATAACGTCG	AATATTACCA	ATGTTTATTT	CGTTATCTGTA	GTGTTTAAAG	TGTTTATTTT
4630	4640	4650	4660	4670	4680
CAITTTTITTC	ACITGCATTTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	TCTTATCATG
GTAATAAATAAG	TGACGTAAGA	TCAACACCAA	ACAGGTTTGA	GTAATATACAT	AGAATAGTAC
4690	4700	4710	4720	4730	4740
TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTTCCTG
AGACATATGG	CAGCTGGAGA	TCGATCTCGA	ACCGCAATTAG	TACCAGTATC	GACAAAGGAC
4750	4760	4770	4780	4790	4800
TGTGAAATTG	TTATCCGCTC	ACAATTCCAC	ACAACATACG	AGCCGGAAGC	ATAAAGTCTA
ACACTTTAAC	AATAGGCGAG	TGTTAAAGGTG	TGTTCATAGC	TCGGCCCTTCG	TATTTTACAT

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FIGURE 191

pD17-hG1b

4810 AAGCC'GCGG TGCC'AAATGA G'GAGCTAAC TCACA'TTAAT 'TGGGTTGCGC TCAC'TGCCCG 4860
TTCCGACCCC ACGGATTACT CACTCGATTG AGTGTAA'TTA ACSCAACGCG AGTGACGGGC
4870 CTTTCCAGTC GGGAAACCTG TCGTGCCAGC TGCA'TTAATG AATCGGCCAA CGCGCGGGGA 4920
GAAAGGTCAG CCTTTGGAC AGCACGGTGC ACGTAAT'TAC TTAGCCCGTT GCGCGCCCCCT
4930 GAGGCGG'TTT GCGTATTGGG CGC'TCTTCCG CTTCCCTCGCT CACTGACTCG CTGCGCTCGG 4980
CTCCGCCCAA CGCATAACCC GCGAGAAGGC GAAGGAGCGA GTGACTGAGC GACGCGAGCC
4990 TCGTTCCGGT GCGGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAATACGG TTATCCACAG 5040
AGCAAGCCGA CGCCGCTCGC CATAGTCGAG TGAGTTTCCG CCATTATGCC AATAGGTGTC
5050 AATCAGGGA TAACGCAGGA AAGAACATGT GAGCAAAAGG CCAGCAAAAG GCCAGGAACC 5100
TTAGTCCCTT ATTGCGTCTT TTCTTGTTACA CTCGTTTTC GGTCTTTTC CGGTCCCTTG
5110 GTAAAAGGC CGCGTTGCTG GCGTTTTC ATAGGCTCCG CCCCCCTGAC GAGCATCACA 5160
CATTTTCCG GCGCAACGAC CGCAAAAGG TATCCGAGGC GGGGGGACTG CTCGTAGTGT
5170 AAAATCGACG CTCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAGA TACCAGGCGT 5220
TTT'TAGCTGC GAGTTTCACTC TCCACCGCTT TGGGCTG'TCC TGATATT'TCT ATGGTCCGCA
5230 TTCCCCCTGG AAGTCCCTC GTGGGCTCTC CTGTTCCGAC CCGTCCGCTT ACCGGATACC 5280
AAGGGGACC TTTCAGGGAG CACGCGAGG GACAAGGCTG GGACGGCGAA TGGCCCTATGG
5290 TGTCCGCCCTT TCTCCCTTCG GGAAGCGTGG CGCTTCTCA ATGCTCACGC TGTAGGTATC 5340
ACAGGCGGAA AGAGGGAAGC CCTTCGCACC GCGAAAGAG'T TACGAGTGC ACATCCATAG
5350 TCAGTTCGGT GTAGGTCGTT CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCCGTTACGC 5400
AGTCAAGCCA CATCCAGCAA GCGAGGTTCC ACCCGACACA CGTCTTGGG GGGCAAGTCG

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FIGURE 19J

pD17-hG1b

5410 CCGACCGCTG CGCCTTATCC GGTAACTATC GTCTTGAGTC CAACCCGGTA AGACACGACT 5460
GGCTGGCGAC CCGGAATAGG CCATTGATAG CAGAACTCAG GTTGGGCCAT TCCTGTCTGA
5470 TATCGCACT GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG 5520
ATAGCGGTGA CCGTCGTCCG TGACCATGTG CCTAATCGTC TCGCTCCATA CATCCGCCAC
5530 CTTACAGAGTT CTTGGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGGACA GTATTTGGTA 5580
GATGTCACAA GAACTTCACC ACCGGATTGA TGCCGATGTG ATCTTCCTGT CATAAACCAT
5590 TCTGCGCTCT GCTGAAGCCA GTTACCCTTCG GAAAAAGAGT TGGTAGCTCT TGATCCGGCA 5640
AGACCGGAGA CGACTTCGGT CAATGGAAGC CTTTTCCTCA ACCATCGAGA ACTAGGCCGT
5650 AACAAACCCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA 5700
TTGTTTGGTG GCGACCATCG CCACCAAAAA AACAAACGTT CGTCGTCTAA TGCGCGTCTT
5710 AAAAAGGATC TCAAGAAGAT CCTTTGATCT TTCTACGGG GTCTGACGCT CAGTGGAAACG 5760
TTTTTTCCTAG AGTTCTCTA AATTCCTTAA GGAAACTAGA AAAGATGCCC CAGACTGCGA GTCACCTTGC
5770 AAAACTCAGC TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC ACCTAGATCC 5820
TTTTTGAGTCC AATTCCCTAA AACCAGTACT CTAATAGTCT TTCTTAGAAG TGGATCTAGG
5830 TTTTAAATTA AAAATGAAGT TTTAAATCAA TCTAAAAGTAT ATATGAGTAA ACTTGGTCTG 5880
AAAATTTAAT TTTTACITCA AAATTAGTT AGATTTCATA TATACTCATT TGAACCCAGAC
5890 ACAGTTACCA ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA TTTCTGTTCT 5940
TGTCATATGGT TACGAATTAG TCACTCCGCTG GATAGAGTCG CTAGACAGAT AAAGCAAGTA
5950 CCATAGTTGC CTGACTCCCC GTCTGTGAGA TAACTACCAT ACGGGAGGGC TTACCATCTG 6000
GGTATCAACC GACTGAGGGG CAGCACATCT ATTGATCTTA TGGCCCTCCCG AATGGTAGAC

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FIGURE 19K

pD17-hG1b

6010 6020 6030 6040 6050 6060
 GCCCCAGTGC TGCAATGATA CCGCGAGACC CAGGTCACC GGCCTCCAGAT TTATCAGCAA
 CCGGGTCACG ACGTTACTAT GCGCTCTGG GTCCGAGTGG CCGAGGTCTA AATAGTCGTT

 6070 6080 6090 6100 6110 6120
 TAAACCAAGCC AGCCGGAAAG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCCTCCA
 ATTGTGTCGG TCGGCCCTTCC CCGCTCGCGT CTTCAACCAGG ACGTTGAAAT AGCGGAGGT

 6130 6140 6150 6160 6170 6180
 TCCAGTCTAT TAATTGTTC CCGGAAGCTA GAGTAAGTAG TTCCGCCAGTT AATAGTTTGC
 AGGTCAGATA ATTAACAACG GCCCTTCGAT CTCATTTCATC AAGCGGTCAA TTATCAAACG

 6190 6200 6210 6220 6230 6240
 GCAACGTTGT TGCCATTGCT ACAGGCATCG TGGTGTCAAG CTCTGTCGTTT GGTATGGCTT
 CGTTGCAACA ACGGTAACGA TGTCCTAGC ACCACAGTGC GAGCAGCAAA CCATACCGAA

 6250 6260 6270 6280 6290 6300
 CATTCACTC CGGTTCCCAA CGATCAAGGC GAGTTACATG ATCCCCCATG TTGTGCAAAA
 GTAAGTCGAG GCCAAGGTT GCTAGTTCCG CTCAATGTAC TAGGGGGTAC AACACGTTTT

 6310 6320 6330 6340 6350 6360
 AAGCGGTTAG CTCTTTCGGT CCTCCGATCG TTGTCAGAAG TAAGTTGGCC GCAGTGTAT
 TTTCGCCAATC GAGGAAGCCA GGAGGCTAGC AACAGTCTC ATTCAACCCG CGTCACAATA

 6370 6380 6390 6400 6410 6420
 CACTCATGGT TATGGCAGCA CTGCATTAAT CTCTTACTGT CATGCCATCC GTAAGATGCT
 GTGAGTACCA ATACCGTCGT GACGTATTAA GAGAATGACA GTACGGTAGG CATTCACGA

 6430 6440 6450 6460 6470 6480
 TTTCTGTGAC TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG CGGCGACCGA
 AAAGACACTG ACCACTCATG AGTTGGTTCA GTAAGACTCT TATCACATAC GCCCGTGGCT

 6490 6500 6510 6520 6530 6540
 GTTGTCTTTG CCGGGCGTCA ATACGGGATA ATACCGGCC ACATAGCAGA ACTTTAAAG
 CAACGAGAAC GGGCCGCAGT TATGCCCTAT TATGGCGCGG TGTATCGTCT TGAATTTTC

 6550 6560 6570 6580 6590 6600
 TGCTCATCAT TGGAAACGT TCTTCGGGGC GAAACTCTC AAGGATCTTA CCGCTGTGA
 ACGAGTACTA ACCTTTTCGA AGAAGCCCCG CTTTGTAGAG TTCCTAGAAAT GCCGCAACT

FIGURE 19L

pD17-hG1b

6610 GATCCAGTTC 6620 GATGTAACCC 6630 ACTCGTGCAC 6640 CCAACTGATC 6650 TTCAGCATCT 6660 TTACTTTTCA
CTAGGTCAAG CTACATTGGG TGAGCACGTG GGTGACTAG AAGTCGTAGA AAATGAAAGT
6670 CCACGGTTC 6680 TGGGTGAGCA 6690 AAAACAGGAA 6700 GGCAAAATGC 6710 CGCAAAAAG 6720 GGAATAAGGG
GGTCGCAAG ACCCACTCGT TTTTGTCTT CCGTTTACG GCGTTTTTC CTTATTCCC
6730 CGACACGAA 6740 ATGTTGAATA 6750 CTCATACTCT 6760 TCCTTTTTCA 6770 ATATTATTGA 6780 AGCATTTATC
GCTGTGCCCT TACAACCTTAT GAGTATGAGA AGGAAAAGT TATAATAACT TCGTAAATAG
6790 AGGGTTATTG 6800 TCCTATGAGC 6810 GGATACATAT 6820 TTGAATGTAT 6830 TTAGAAAAAT 6840 AAACAAATAG
TCCCAATAAC AGAGTACTCG CCTATGTATA AACTTACATA AATCTTTTTA TTTGTTTTATC
6850 GGGTCCGCG 6860 CACATTTCCC 6870 CGAAAAGTGC 6880 CACCTGACGT 6890 CGACGGATCG 6900 GGAGATCTGC
CCCAAGGCGC GTGTAAAGGG GCTTTTACG GTGGACTGCA GCTGCCTAGC CCTCTAGACG
6910 TAGGTGACCT 6920 GAGGCGCGCC 6930 GGCTTCGAAT 6940 AGCCAGAGTA 6950 ACCTTTTTTTT 6960 TTAATTTTTAT
ATCCACTGGA CTCCGCGCGG CCGAAGCTTA TCGGTCTCAT TGGAAAAAA AATTAAAAATA
6970 TTTTATTTTAT 6980 TTTTGAGATG 6990 GAGTTTGGCG 7000 CCGATCTCCC 7010 GATCCCCCTAT 7020 GGTCGACTCT
AANTAAAAATA AAAACTCTAC CTCAAACCCG GGTAGAGGG GTAGGGGATA CCAGCTGAGA
7030 CAGTACAATC 7040 TGCTCTGATG 7050 CCGCATAGTT 7060 AAGCCAGTAT 7070 CTGCTCCCTG 7080 CTTGTGTGTT
GTCATGTTAG ACGAGACTAC GCGGTATCAA TTCCGGTCATA GACGAGGGAC GAACACACAA
7090 GGAGGTCGCT 7100 GAGTAGTGGC 7110 CGAGCAAAAT 7120 TTAAGCTACA 7130 ACAAGGCAAG 7140 GCTTGACCGA
CCTCCAGCGA CTCATCACGC GCTCGTTTTTA AATTCGATGT TGTTCGGTTC CGAACTGGCT
7150 CCAATTGCATG 7160 AAGAACTCTG 7170 TTAGGGTTAG 7180 GCGTTTTCGG 7190 CTGCTTCGCG 7200 ATGTACGGGC
GTTAACGTAC TTCTTAGACG AATCCCAATC CGCAAAACCG GACGAAGCGC TACATGCCCG

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FIGURE 19M

pD17-hG1b

7210 CAGATATACG CGTTGACATT GATTATTGAC TAGTTATTAA TAGTAATCAA TTACGGGGTC 7260
GTCATATATGC GCAACTGTAA CTAATAACTG ATCAATAATT ATCATTAGTT AATGCCCCAG
7270 ATTAGTTTAT AGCCCATATA TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC 7320
TAATCAAGTA TCGGGTATAT ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG
7330 TGGCTGACCG CCCAAGGACC CCCGCCCAAT GACGTCAATA ATGACGTATG TTCCCATAGT 7380
ACCGACTGGC GGGTGTCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA
7390 AACCCCAATA GGGACTTTC ATTGACGTCA ATGGGTGGAC TATTACGGT AAAC TGCCCA 7440
TTGGCGTTAT CCTGAAAGG TAAC TGCAGT TACCCACCTG ATAAATGCCA TTGACGGGT
7450 CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCC CCTATTGACG TCAATGACGG 7500
GAACCGTCAT GTAGTTTACA TAGTATACGG TTTCATGCGG GGATAACTGC AGTTACTGCC
7510 TAAATGGCC GCCTGGCATT ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA 7560
ATTTACCCGG CGGACCGTAA TACGGGTCTAT GTACTGGAAT ACCCTGAAAG GATGAACCGT
7570 GTACATCTAC GTATTAGTCA TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA 7620
CATGTAGATG CATAATCAGT AGCGATAATG GTACCACCTAC GCCAAAACCG TCATGTAGTT
7630 TGGGCGTGA TAGCGGTTTG ACTCACGGGG ATTTCCCAAGT CTCCACCCCA TTGACGTCAA 7680
ACCCGCACCT ATCGCCNAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGT AAC TGCAGTT
7690 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCTGA ACAACTCCGC 7740
ACCCTCANAC AAAACCGTGG TTTTAGTTGC CCTGAAAAGT TTACAGCAT TGT TGAAGCGG
7750 CCCATTGACG CAAATGGCG GTAGCGGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT 7800
GGGTAAC TGC GTTACCCCGC CATCCGCACA TGCCACCCCTC CAGATATATT CGTCTCGAGA

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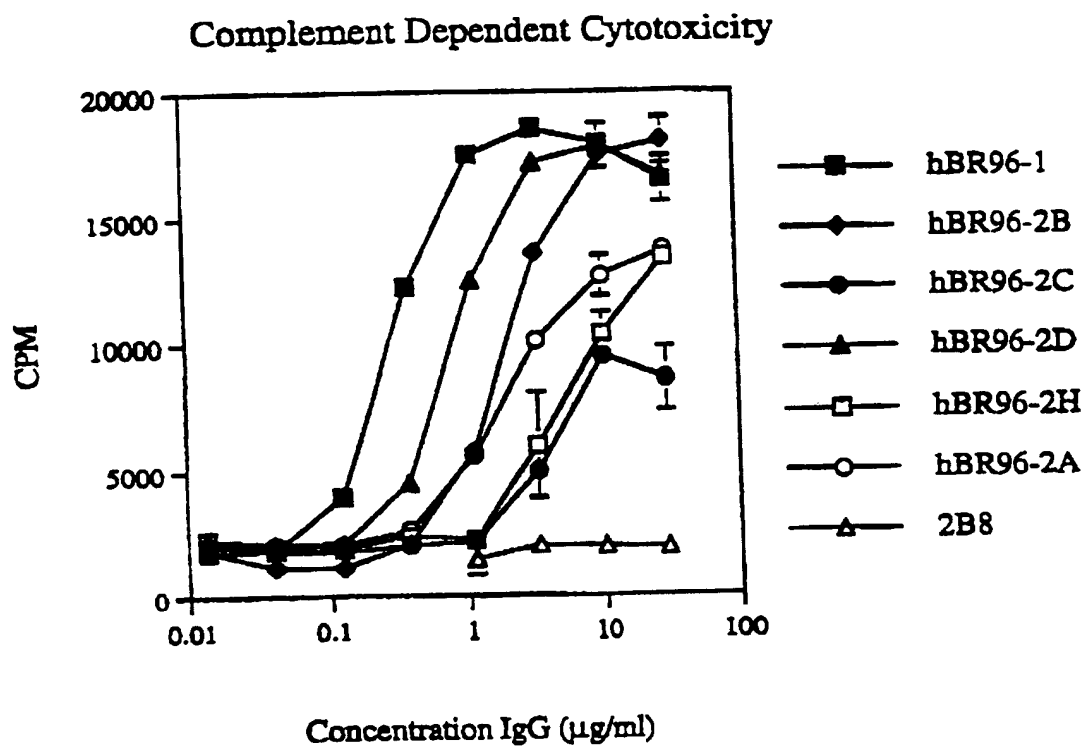
FIGURE 19N

pD17-hG1b

7810	7820	7830	7840	7850	7860
CTGGCTAACT	AGAGAACCCA	CTGCTTACTG	GCATTATCGAA	ATTAAATACGA	CTCACTATATAG
GACCGATTGA	TCTCTTGGGT	GACGAATGAC	CGAATAGCCT	TAAATTATGCT	GAGTGATATC
7870	7880				
GGAGACCCAA	GCTT				
CCTCTGGGTT	CGAA				

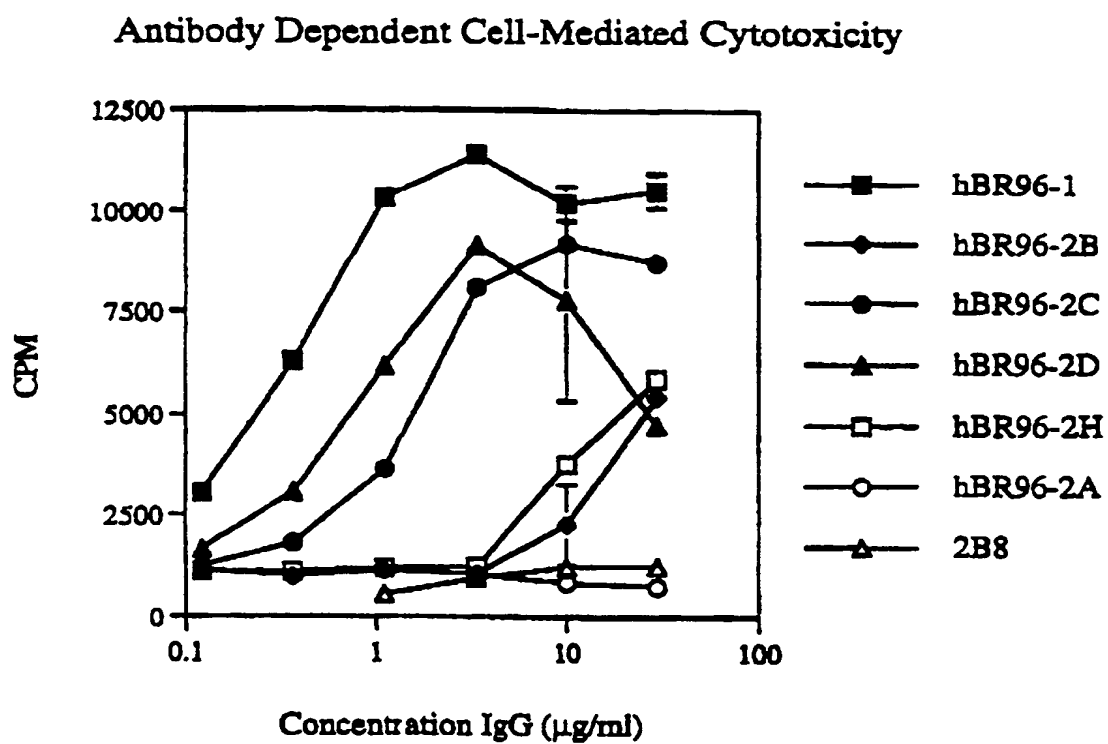
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FIGURE 20



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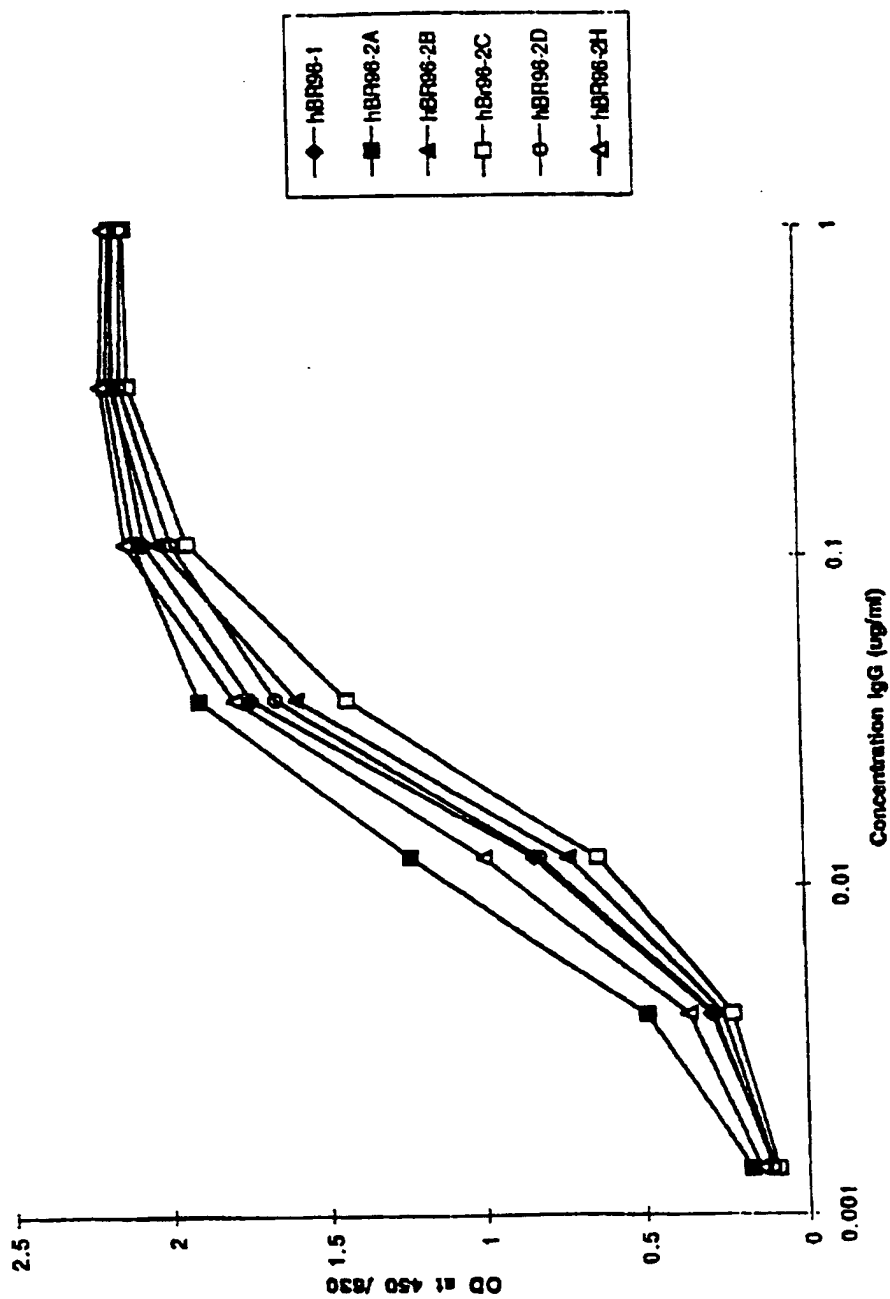
FIGURE 21



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FIGURE 22

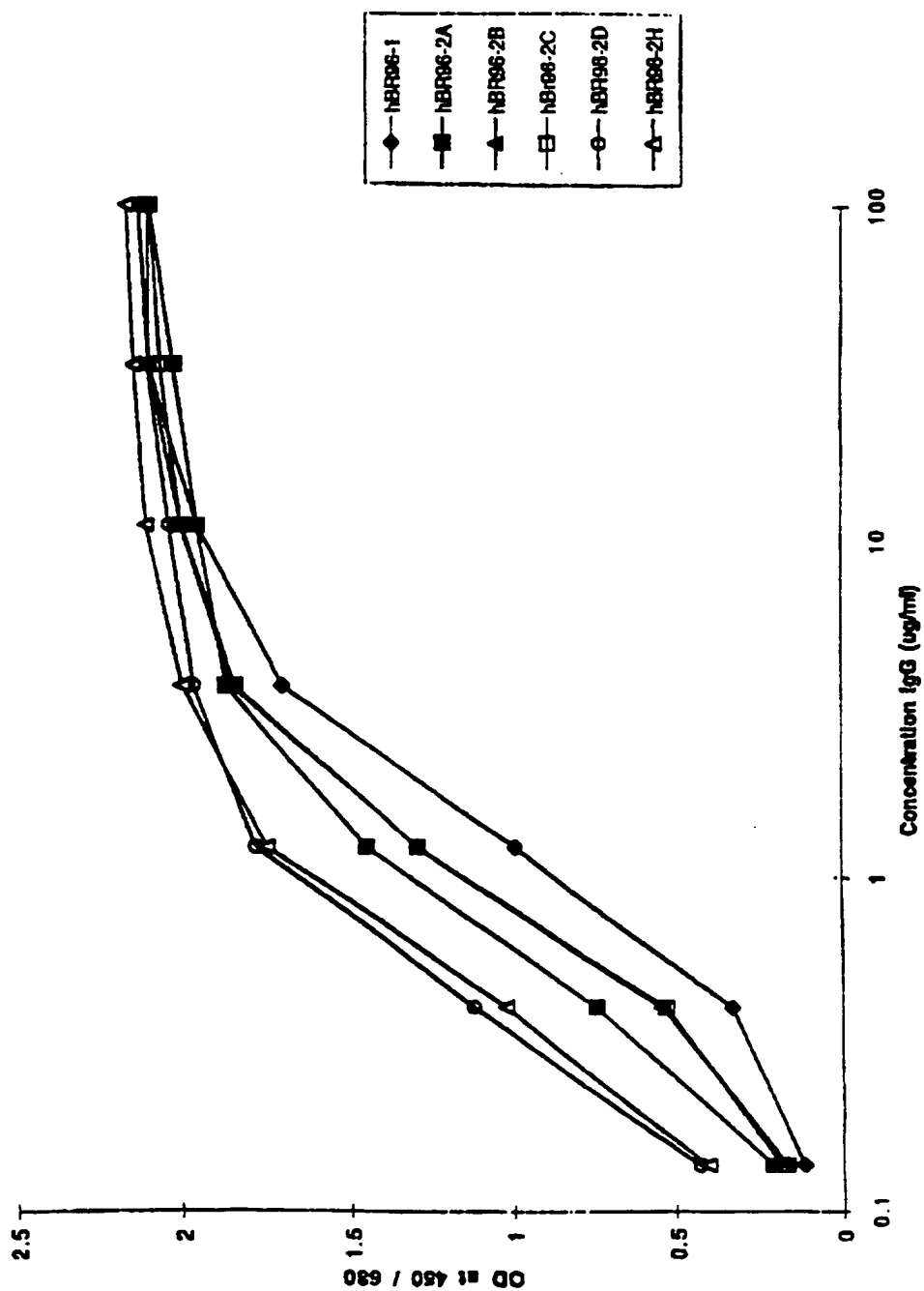
Binding activity of hBR96-2 constant region mutants on LoY-HSA



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FIGURE 23

Binding activity of hBR96-2 constant region mutants on LNFPII-BSA



51156

Figure 24

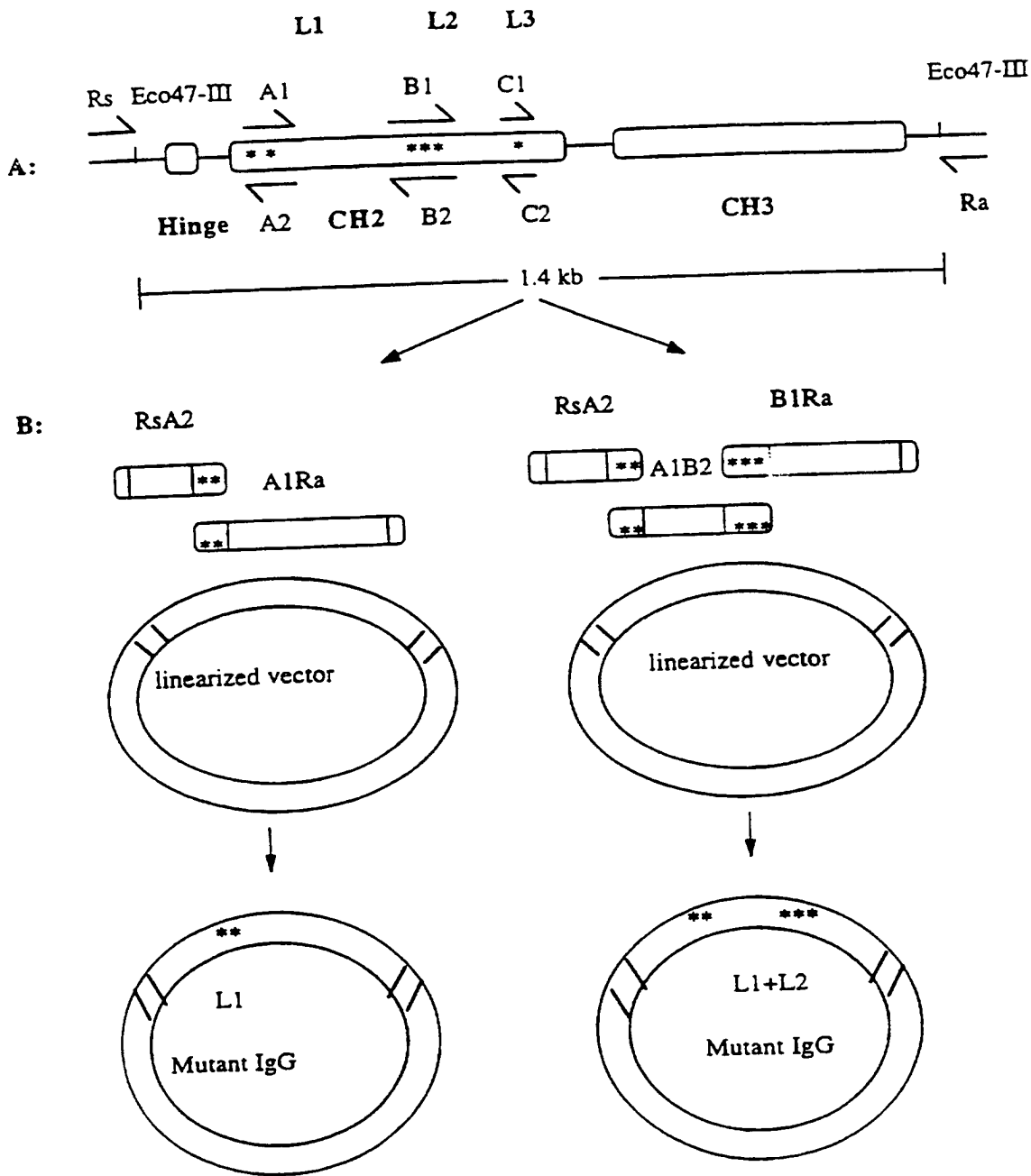


Figure 25

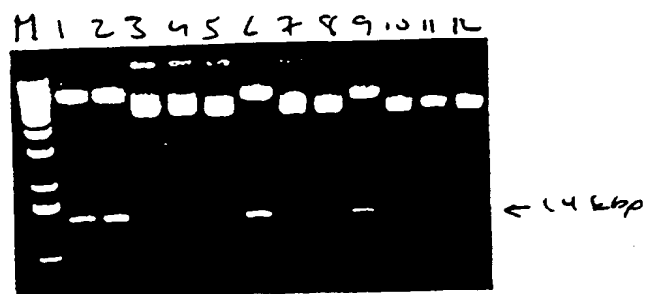


Figure 26

hBR96-2 Heavy Chain Variable Region (VH)

1 11 21 31 41
 EVQLVESGGG LVQPGGSLRL SCAASGFPS DYMYWVRQA PGKGLEWVSY
 51 61 71 81 91
 ISQDGDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL
 101 111
 ADGAWFAYWG QGTLTVSS

human IgG1 constant

CH1
 A STKGPSVFPL APSSKSTSGG TAALGCLVKD
 YFPEFVTVSW NSGALTSGVH TTPAVLQSSG LYSLSVTV PSSSLGTQTY
 ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CH2 333 337 SVFLFPPKPK
 DTLNISRTPE VTCVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
 TYRVVSVLTV LHQDWLNGKE 318 320 322 YQKQVSNKAL PAPIEKTISK AKGQPREPQV
 YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTFPVL
 DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVN HEALHNHYTQ KSLSLSPGK

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Figure 27

hBR96-2A: Heavy Chain Variable Region (VH)

1 11 21 31 41
 EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYMYWVRQA PGKGLEWVS
 51 61 71 81 91
 ISQDGDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL
 101 111
 ADGAWFAYWG QGTLTVSS

hBR96-2A: Human Heavy Chain IgG1 Constant Region Δ CH2

A STKGPSVFPL APSSKSTSGC TAALGCLVKD YFPEPVTVSW NSGALTSGVH
 TTPAVLQSSG LYSLSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKVEPK
 SCDKTHTCPP CP GQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA
 VEWESNGQPE NNYKTTTPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
 HEALHNHYTC KSLSLSPGK

Figure 28

This sequence is the chi BR96 IgG1 with CH2 deleted.

VH
1 EVNLVESGGG LVQPGGSLKV SCVTSGFTPS DYMYWVRQT PEKRLWVAY
51 ISQGGDITDY PDTVRGRFTI SRDIAKNTLY LQMSRLKSED TAMYTCARGL
101 DDGAWFAYWG QGTLVTVSVA ^{CH1}STKGPSVFPL APSSKSTSGG TAALGCLVKD
151 YFPEPVTVSW NSGALTSGVH TFFAVLQSSG LYSLSVVTV PSSSLGTQTY
201 ICNVNHHKPSN TKVDKKVEPK SCDKTHTCPP ^{CH3}CHGQPREPQV YTLPPSRDEL
251 TRNQVSLTCL VKGFYPSDIA VENESNGQPE NNYKTTPFVL DSDGSFFLYS
301 KLTVDKSRWQ QGNVFSCSVN HEALHNHYTQ KSLSLSPGK

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/13562

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 26,27

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 26 represents a method of detection/diagnosis and refers forward to claim 30, which represents a method of treatment. Claim 27 refers to a method in claim 24; however, in claim 24 a product is claimed, not a method.

Remark : Although claims 1-22, 25, 28-32 and 34-36 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/13562

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 699756 A	06-03-96	AU 2834995 A	15-02-96
		CA 2155397 A	05-02-96
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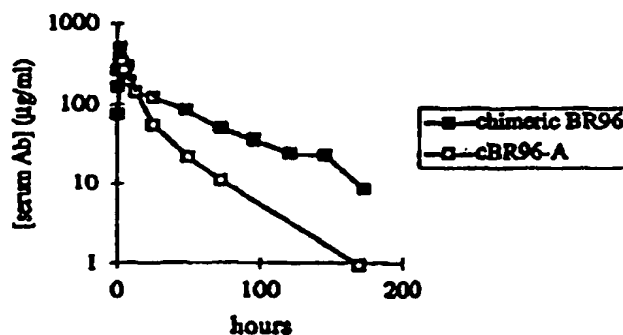
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60/023,033 2 August 1996 (02.08.96) US(71) Applicant: BRISTOL-MYERS SQUIBB COMPANY
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claims and to be republished in the event of the receipt of
amendments.*(54) Title: A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY RESULTING FROM THE USE OF
IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSISPlasma clearance in high LeY expressing dogs chimeric versus constant region mutant of
cBR96-2.

(57) Abstract

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an immunoglobulin or Ig fusion protein molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by inactivation of at least a portion of the constant region.

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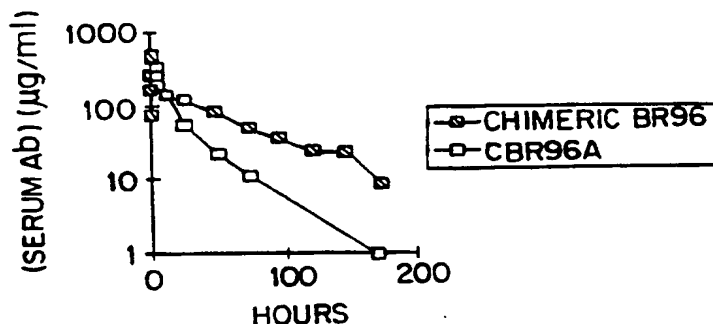
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5 **A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED
TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN
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10 Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

15 **TECHNICAL FIELD OF THE INVENTION**

The present invention relates to methods for inhibiting or reducing immunoglobulin-induced toxicity resulting from therapy or in vivo diagnosis. Specifically, in lieu of using unmodified antibodies or recombinant binding proteins for in vivo use, the
20 invention provides the use of modified antibodies or recombinant binding proteins which have been structurally altered in the constant domain so that upon administration immunoglobulin-induced toxicity is reduced or inhibited.

BACKGROUND OF THE INVENTION

25

Over the years investigators have attempted to harness the immune system for therapeutic use. Immunoglobulin (Ig) molecules which constitute an important part of the immune system are of great interest because they (1) react with a diverse family of ligands, (2) possess different effector functions and (3) are of great
30 biological importance. Despite its potential, a persistent problem with

immunoglobulin immunotherapy has been, among other problems, the toxic effect to normal cells of using antibodies which recognize both normal and diseased cells. This problem is far-reaching because the majority of antibodies presently available recognize a target located on both normal and diseased cells (Slavin-Chiorini, et al.,
5 Int. J. Cancer 53: 97-103 (1993)).

The constant region can promote cell death through antibody dependent cell mediated cytotoxicity (ADCC) or by complement dependent cytotoxicity (CDC). Despite the deletion of portions of the constant region, particularly the CH₂ domain,
10 the antigen binding function can be retained (D. Yelton, M. Scharf, Mutant monoclonal antibody with alterations in biological functions, J. Exp. Methods 156:1131-1148 (1982)).

Others have generated a CH₂-deleted antibody (Mueller et al., Proc. Natl. Acad. Sci.
15 USA 87: 5702-5705 (1990)). Their findings provide that the CH₂-deleted antibody was cleared from the blood of tumor-bearing mice much faster than the corresponding intact antibody. Other in vivo findings also confirmed that a CH₂-deleted antibody, designated ch14.18DCH2, is a potentially useful reagent for radioimmunodetection of human tumors because of its reduced immunogenicity,
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Generally, whole antibody molecules are composed of two heavy (H) and two light (L) chains which are held together by covalent bonds (disulfide) and non-covalent
25 interactions. Each chain contains a variable region (V) and a constant region (C). The variable regions at the amino termini of the two chains form the antigen binding region. The constant region of the H chain has three components or domains. Occasionally, the first constant region domain (CH₁) interacts with the C region of the L chain through hydrophobic interactions and generally a disulfide bond,

depending on isotype. The next C region stretch is the hinge-acting disulfide bond stably introduced between two H chains. The second constant region domain (CH₂) is adjacent to the hinge region. CH₂ contains sequences important for effector functions of the antibody, such as the sequences responsible for complement
5 fixation, and Fc receptor binding. The third constant region domain (CH₃) is located at the carboxyl terminus of the H chain, and is considered to play an important role in H chain assembly as well as some C region functions.

Today many antibodies in clinical trials are directed against tumor associated
10 antigens. Most tumor associated antigens are not tumor specific but are also generally found on the cell surface of some normal, non-tumorigenic cells. The clinical use of some antibodies directed against tumor associated antigens are limited because of the toxicity associated with their use. Therefore, there is a need for methods for inhibiting toxicity associated with immunoglobulin use in the field of
15 disease therapy (e.g., therapy for tumors, kidney disease, and the like) and in vivo diagnosis.

We addressed this need by discovering methods for inhibiting or reducing toxicity to normal cells generally associated with immunoglobulin immunotherapy or in vivo
20 diagnosis, wherein the immunoglobulin recognizes both diseased and normal cells. Our discovery involves generating immunoglobulin molecules or Ig fusion proteins having structurally altered constant regions which inhibit or reduce immunoglobulin-induced toxicity.

25 SUMMARY OF THE INVENTION

The present invention provides methods for inhibiting immunoglobulin-induced toxicity by using known immunoglobulin or Ig fusion protein molecules which are structurally altered in their constant regions so that the resulting structurally altered

immunoglobulin or Ig fusion protein molecules exhibit reduced or inhibited toxicity in vivo compared to their original unmodified counterparts.

5 Structural alteration of the constant region may be effected in a number of ways as long as it results in reducing or inhibiting immunoglobulin-induced toxicity.

In accordance with the practice of the invention, structural alteration of the constant region is effected by deletion of the entire constant region. In another embodiment, only the CH₂ domain is deleted. In another embodiment, only that portion of the
10 CH₂ domain that binds the Fc receptor is deleted. In yet another embodiment, only that portion of the CH₂ domain that binds the complement component C1q is deleted. Alternatively, in another embodiment, multiple deletions in discrete Fc receptor and complement component binding domains are effected.

15 Alternatively, structural alteration is effected by single or multiple mutations in the CH₂ domain such as amino acid insertions and substitutions. The mutation or mutations must result in inhibiting immunoglobulin-induced toxicity. By way of example, the amino acids in multiple toxicity associated domains in the constant region can be altered so as to render the constant region unable to mediate a ADCC
20 response or activate complement thereby inhibiting immunoglobulin induced toxicity resulting from immunotherapy. Alternatively, multiple amino acids in a single toxicity associated domain in the constant region can be altered.

Further alternatively, structural alteration can be effected by isotype switching
25 resulting in an altered immunoglobulin molecule that either does not induce toxicity or induces some limited toxicity but does not cause a harmful effect. For example, isotype switching can result in the constant region being unable to mediate a CDC or ADCC response or some other activity which mediates toxicity.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a line graph showing plasma clearance in high Le^Y expressing dogs using chimeric BR96 versus constant region mutant of cBR96-2.

5

Figure 2 is a schematic diagram of a plasmid designated pTWD-cJVK.L1 including the chimeric (c)BR96-light chain (SEQ ID NO. 11).

10 Figure 3 is a schematic diagram of a plasmid designated pD16hJ1.L1 including the human (h)BR96-light chain (SEQ ID NO. 13).

Figure 4 is a schematic diagram of a plasmid, designated pD17-hJm14-dCH2.H1, of hBR96-2A (i.e., human mutant BR96 having the H1, H2, and H3 mutations and the CH_2 deletion (PCT Application No. 95/305444, published March 6, 1996)).

15

Figure 5 is a schematic diagram of a plasmid, designated pD17-cJ-dCH2.H1, of cBR96-A (SEQ ID NO. 10) (i.e., chimeric BR96 having the CH_2 deletion (PCT Application No. 95/305444, published March 6, 1996)).

20 Figure 6 is a schematic diagram of a plasmid, designated pD17-cJ.H1, of cBR96.

Figure 7 is a line graph showing the results of an ELISA assay of (1) hBR96-2A-Dox to Le^Y (closed diamond), (2) hBR96-2A to Le^Y (96:0006A2 R/A)(closed square), (3) hBR96-2A to Le^Y (96:0006B R/A)(closed triangle), and BR96-Dox to Le^Y (X).

25

Figure 8 is a line graph showing the results of an ELISA assay of (1) BR96-A-Dox to Le^Y (closed diamond), (2) chiBR96 to Le^Y (closed square), (3) cBR96-A to Le^Y (96:0003 R/A)(closed triangle), and cBR96-Dox to Le^Y (X).

Figures 9a-c are schematic diagrams showing the steps for deleting a CH₂ domain.

Figures 10a-c are schematic diagrams showing the construction of BR96 IgG1 CH₂
5 domain point mutations.

Figure 11 is a schematic diagram showing the construction of the pNg1/14 vector.

Figure 12 is a schematic diagram showing the construction of pD17-hBR96-2.
10

Figure 13 is a schematic diagram showing the construction of pD17-hJm14-
dCH2.H1.

Figure 14 is the nucleic acid sequence of pD17-cJ-dCH2.H1, the plasmid shown in
15 Figure 5, chimeric BR96 having the CH₂ deletion.

Figure 15 is a line graph showing the results of an ELISA assay comparing whole
chiBR96 and deleted CH₂ chiBR96 on Le^y.

20 Figure 16 is a description of the seven structural alterations.

Figure 17 is a schematic diagram of a plasmid designated pD17-hG1b.

Figure 18 is the nucleic acid sequence of pD17-hJm14.H1.
25

Figure 19 is the nucleic acid sequence of pD17-hG1b.

Figure 20 is a line graph showing complement dependent cytotoxicity. In the
legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is

hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b mAb, negative control.

5 Figure 21 is a line graph showing antibody dependent cell-mediated cytotoxicity. In the legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b monoclonal antibody (mAb), negative control.

10

Figure 22 is a line graph showing binding activity of hBR96-2 constant region mutants on LeY-HSA. In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331
15 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

Figure 23 is a line graph showing binding activity of hBR96-2 constant region mutants on LNFPIII-BSA. LNFPIII is a lacto-N-fucopentasose, a Lewis X
20 trisaccharide with an additional lactose spacer (V Labs, Covington, LA). In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

25

Figures 24A and 24B provide a strategy for introducing multiple mutations by RPCR. (A) Diagram of the 1.4 kpb IgG heavy chain region showing the hinge CH₂ and CH₃ domains as boxed regions. Site-specific mutations to be introduced into CH₂ positions L1, L2, and L3 are encoded by complementary sets of mutant PCR

primers (A1 and A2; B1 and B2; and C1 and C2). The asterisks (*) indicate the number of amino acid changes introduced at each L position. The two PCR primers, Rs (Recombination -sense) and Ra (Recombination-antisense), flank the Eco-47-III restriction sites and mediate homologous recombination with vector ends. The 3' ends of the oligonucleotides are represented by arrowheads. (B) A three-way homologous recombination event between fragments RsA2, A1Ra and the linearized vector produces the L1 mutant IgG. Two distally located sets of mutations (L1 and L2) are simultaneously introduced by increasing the number of recombining PCR products as is shown in the four-way recombination of RsA2, A1B1, B1Ra with vector.

Figure 25 is a gel showing Eco-47-III restriction endonuclease analysis of DNAs prepared from colonies generated by multiple PCR fragment RPCR. Lane M: 1kb ladder DNA marker (GIBCO/BRL Life Science Technology). Lanes 1-12: Twelve randomly selected colonies resulting from quadruple homologous recombination events were used to prepare plasmid and digested with Eco47-III. Clones 1, 2, 6 and 9 contain the fully assembled 1.4 kpb insert.

Figure 26 provides the amino acid sequence for hBR96-2 heavy-chain variable region and the human IgG1 constant region.

Figure 27 provides the amino acid sequence for hBR96-2A heavy-chain variable region and the human IgG1 constant region.

Figure 28 provides the amino acid sequence for chi BR96 heavy-chain variable region and the human IgG1 constant region without the CH₂ domain.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

5 As used herein the term "inhibiting immunoglobulin-induced toxicity" means to reduce or alleviate symptoms generally associated with toxicity caused by immunoglobulin or Ig fusion protein therapy, e.g., toxicity mediated by effector functions of the Fc receptor. For example, BR96 antibody recognizes and binds BR96 antigen which is found at some levels in the gastrointestinal tract and at
10 elevated levels in tumors (as compared to the gastrointestinal tract of normal tissues). The binding of BR96 antibody to BR96 antigen in vivo causes symptoms associated with gastrointestinal toxicity. These symptoms include rapid onset of vomiting, often with blood, and nausea. In humans the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach.

15 The pathology of the wound is limited and resolves. However, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity. For highly elevated levels of other antigens found in the central nervous system (CNS), liver, and other locations, the toxicity will be characterized by
20 symptoms other than those described above.

As used herein the term "immunoglobulin molecule" can be produced by B cells or be generated through recombinant engineering or chemical synthetic means. Examples of immunoglobulin molecules include (1) antibodies, e.g., polyclonal and
25 monoclonal antibodies, chimeric or humanized, and (2) recombinant Ig containing binding proteins, e.g., Ig fusion proteins. Recombinant Ig containing binding proteins include cell surface proteins, e.g., CD antigens (in one embodiment, CTLA4), to which an Ig tail is joined.

As used herein the terms "structurally altered" or "structural alteration" means manipulating the constant region so that the resulting molecule or protein exhibits a diminished ability to induce toxicity. Structural alteration can be by chemical modification, proteolytic alteration, or by recombinant genetic means. Recombinant
5 genetic means may include, but is not limited to, the deletion, insertion and substitution of amino acid moieties.

As used herein the terms "multiple toxicity associated domains" means more than one discrete toxicity associated domain. As there appear to be at least two toxicity
10 associated domains in the immunoglobulin molecule, one roughly localized to amino acids 231-238 and another roughly localized to amino acids 310-331, an example of the structural alteration of multiple toxicity associated domains comprises the insertion, substitution or deletion of amino acid residues in both of these domains. This definition excludes structural alterations targeting a single toxicity associated
15 domain.

Merely by way of example, the constant region of the immunoglobulin molecule can be structurally altered so that the molecule no longer mediates a CDC or ADCC response. However, the methods of the invention encompasses the use of
20 structurally altered immunoglobulin molecules regardless of whether it mediates a CDC or ADCC response. The underlying requirement is that the altered molecule must inhibit immunoglobulin-induced toxicity.

Structural alteration can be effected in a number of ways. For example, structural
25 alteration can be effected by deletion of the entire constant region.

Alternatively, structural alteration can be effected by deletion of the entire CH₂ domain of the constant region. In this instance, deletion of the entire CH₂ domain may render the molecule unable to (1) bind an Fc receptor thereby eliminating the

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10 In accordance with the practice of the invention, structural alteration of the constant region is effected by deletion of the entire constant region. In another embodiment, only the CH₂ domain is deleted. In another embodiment, only that portion of the CH₂ domain that binds the Fc receptor is deleted. In yet another embodiment, only that portion of the CH₂ domain that binds the complement component C1q is deleted. Alternatively, in another embodiment, multiple deletions in discrete Fc receptor and complement component binding domains are effected.

15 Alternatively, structural alteration is effected by single or multiple mutations in the CH₂ domain such as amino acid insertions and substitutions. The mutation or mutations must result in inhibiting immunoglobulin-induced toxicity. By way of example, the amino acids in multiple toxicity associated domains in the constant region can be altered so as to render the constant region unable to mediate a ADCC response or activate complement thereby inhibiting immunoglobulin induced toxicity resulting from immunotherapy. Alternatively, multiple amino acids in a single toxicity associated domain in the constant region can be altered.

25 Further alternatively, structural alteration can be effected by isotype switching resulting in an altered immunoglobulin molecule that either does not induce toxicity or induces some limited toxicity but does not cause a harmful effect. For example, isotype switching can result in the constant region being unable to mediate a CDC or ADCC response or some other activity which mediates toxicity.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a line graph showing plasma clearance in high Le^y expressing dogs using chimeric BR96 versus constant region mutant of cBR96-2.

5

Figure 2 is a schematic diagram of a plasmid designated pTWD-cJVK.L1 including the chimeric (c)BR96-light chain (SEQ ID NO. 11).

Figure 3 is a schematic diagram of a plasmid designated pD16hJ1.L1 including the human (h)BR96-light chain (SEQ ID NO. 13).

10

Figure 4 is a schematic diagram of a plasmid, designated pD17-hJm14-dCH2.H1, of hBR96-2A (i.e., human mutant BR96 having the H1, H2, and H3 mutations and the CH₂ deletion (PCT Application No. 95/305444, published March 6, 1996)).

15

Figure 5 is a schematic diagram of a plasmid, designated pD17-cJ-dCH2.H1, of cBR96-A (SEQ ID NO. 10) (i.e., chimeric BR96 having the CH₂ deletion (PCT Application No. 95/305444, published March 6, 1996)).

Figure 6 is a schematic diagram of a plasmid, designated pD17-cJ.H1, of cBR96.

20

Figure 7 is a line graph showing the results of an ELISA assay of (1) hBR96-2A-Dox to Le^y (closed diamond), (2) hBR96-2A to Le^y (96:0006A2 R/A)(closed square), (3) hBR96-2A to Le^y (96:0006B R/A)(closed triangle), and BR96-Dox to Le^y (X).

25

Figure 8 is a line graph showing the results of an ELISA assay of (1) BR96-A-Dox to Le^y (closed diamond), (2) chiBR96 to Le^y (closed square), (3) cBR96-A to Le^y (96:0003 R/A)(closed triangle), and cBR96-Dox to Le^y (X).

Figures 9a-c are schematic diagrams showing the steps for deleting a CH₂ domain.

Figures 10a-c are schematic diagrams showing the construction of BR96 IgG1 CH₂
5 domain point mutations.

Figure 11 is a schematic diagram showing the construction of the pNg1/14 vector.

Figure 12 is a schematic diagram showing the construction of pD17-hBR96-2.

10 Figure 13 is a schematic diagram showing the construction of pD17-hJm14-dCH2.H1.

Figure 14 is the nucleic acid sequence of pD17-cJ-dCH2.H1, the plasmid shown in
15 Figure 5, chimeric BR96 having the CH₂ deletion.

Figure 15 is a line graph showing the results of an ELISA assay comparing whole
chiBR96 and deleted CH₂ chiBR96 on Le^y.

20 Figure 16 is a description of the seven structural alterations.

Figure 17 is a schematic diagram of a plasmid designated pD17-hG1b.

Figure 18 is the nucleic acid sequence of pD17-hJm14.H1.

25 Figure 19 is the nucleic acid sequence of pD17-hG1b.

Figure 20 is a line graph showing complement dependent cytotoxicity. In the
legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is

hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b mAb, negative control.

5 Figure 21 is a line graph showing antibody dependent cell-mediated cytotoxicity. In the legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b monoclonal antibody (mAb), negative control.

10

Figure 22 is a line graph showing binding activity of hBR96-2 constant region mutants on LeY-HSA. In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331
15 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

Figure 23 is a line graph showing binding activity of hBR96-2 constant region mutants on LNFPIII-BSA. LNFPIII is a lacto-N-fucopentasose, a Lewis X
20 trisaccharide with an additional lactose spacer (V Labs, Covington, LA). In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

25

Figures 24A and 24B provide a strategy for introducing multiple mutations by RPCR. (A) Diagram of the 1.4 kpb IgG heavy chain region showing the hinge CH₂ and CH₃ domains as boxed regions. Site-specific mutations to be introduced into CH₂ positions L1, L2, and L3 are encoded by complementary sets of mutant PCR

primers (A1 and A2; B1 and B2; and C1 and C2). The asterisks (*) indicate the number of amino acid changes introduced at each L position. The two PCR primers, Rs (Recombination -sense) and Ra (Recombination-antisense), flank the Eco-47-III restriction sites and mediate homologous recombination with vector ends. The 3' ends of the oligonucleotides are represented by arrowheads. (B) A three-way homologous recombination event between fragments RsA2, A1Ra and the linearized vector produces the L1 mutant IgG. Two distally located sets of mutations (L1 and L2) are simultaneously introduced by increasing the number of recombining PCR products as is shown in the four-way recombination of RsA2, A1B1, B1Ra with vector.

Figure 25 is a gel showing Eco-47-III restriction endonuclease analysis of DNAs prepared from colonies generated by multiple PCR fragment RPCR. Lane M: 1kb ladder DNA marker (GIBCO/BRL Life Science Technology). Lanes 1-12: Twelve randomly selected colonies resulting from quadruple homologous recombination events were used to prepare plasmid and digested with Eco47-III. Clones 1, 2, 6 and 9 contain the fully assembled 1.4 kpb insert.

Figure 26 provides the amino acid sequence for hBR96-2 heavy-chain variable region and the human IgG1 constant region.

Figure 27 provides the amino acid sequence for hBR96-2A heavy-chain variable region and the human IgG1 constant region.

Figure 28 provides the amino acid sequence for chi BR96 heavy-chain variable region and the human IgG1 constant region without the CH₂ domain.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

5 As used herein the term "inhibiting immunoglobulin-induced toxicity" means to reduce or alleviate symptoms generally associated with toxicity caused by immunoglobulin or Ig fusion protein therapy, e.g., toxicity mediated by effector functions of the Fc receptor. For example, BR96 antibody recognizes and binds BR96 antigen which is found at some levels in the gastrointestinal tract and at
10 elevated levels in tumors (as compared to the gastrointestinal tract of normal tissues). The binding of BR96 antibody to BR96 antigen in vivo causes symptoms associated with gastrointestinal toxicity. These symptoms include rapid onset of vomiting, often with blood, and nausea. In humans the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach.

15 The pathology of the wound is limited and resolves. However, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity. For highly elevated levels of other antigens found in the central nervous system (CNS), liver, and other locations, the toxicity will be characterized by
20 symptoms other than those described above.

As used herein the term "immunoglobulin molecule" can be produced by B cells or be generated through recombinant engineering or chemical synthetic means. Examples of immunoglobulin molecules include (1) antibodies, e.g., polyclonal and
25 monoclonal antibodies, chimeric or humanized, and (2) recombinant Ig containing binding proteins, e.g., Ig fusion proteins. Recombinant Ig containing binding proteins include cell surface proteins, e.g., CD antigens (in one embodiment, CTLA4), to which an Ig tail is joined.

As used herein the terms "structurally altered" or "structural alteration" means manipulating the constant region so that the resulting molecule or protein exhibits a diminished ability to induce toxicity. Structural alteration can be by chemical modification, proteolytic alteration, or by recombinant genetic means. Recombinant
5 genetic means may include, but is not limited to, the deletion, insertion and substitution of amino acid moieties.

As used herein the terms "multiple toxicity associated domains" means more than one discrete toxicity associated domain. As there appear to be at least two toxicity
10 associated domains in the immunoglobulin molecule, one roughly localized to amino acids 231-238 and another roughly localized to amino acids 310-331, an example of the structural alteration of multiple toxicity associated domains comprises the insertion, substitution or deletion of amino acid residues in both of these domains. This definition excludes structural alterations targeting a single toxicity associated
15 domain.

Merely by way of example, the constant region of the immunoglobulin molecule can be structurally altered so that the molecule no longer mediates a CDC or ADCC response. However, the methods of the invention encompasses the use of
20 structurally altered immunoglobulin molecules regardless of whether it mediates a CDC or ADCC response. The underlying requirement is that the altered molecule must inhibit immunoglobulin-induced toxicity.

Structural alteration can be effected in a number of ways. For example, structural
25 alteration can be effected by deletion of the entire constant region.

Alternatively, structural alteration can be effected by deletion of the entire CH₂ domain of the constant region. In this instance, deletion of the entire CH₂ domain may render the molecule unable to (1) bind an Fc receptor thereby eliminating the

molecule's possibility of mediating antibody-dependent cellular cytotoxicity (ADCC), (2) bind C1q, or (3) activate complement.

Alternatively, structural alteration can be effected by deletion of only that portion of
5 the CH₂ domain that binds the Fc receptor or complement.

Further alternatively, a single mutation or multiple mutations such as substitutions and insertions in the CH₂ domain can be made. The underlying requirement of any mutation is that it must inhibit, diminish, or block immunoglobulin-induced toxicity.

10 For example, this can be achieved by mutating the constant region such that the altered molecule is rendered unable to mediate a CDC response or an ADCC response, or to activate complement.

Alternatively, structural alteration can be effected by isotype switching (also known
15 as class switching) so that the altered molecule does not induce toxicity in the subject. In one embodiment, the constant region of the immunoglobulin is structurally altered so that it no longer binds the Fc receptor or a complement component, e.g., switching a molecule's original IgG isotype from IgG1 to IgG4. Isotype switching can be effected regardless of species, i.e., an isotype from a non-
20 human being can be switched with an isotype from a human being (E.D. Finkelman et al. (1990) Annu. Rev. Immunol. 8:303-333; T. Honjo et al. (1979) Cell 18: 559-568; T. Honjo et al. In "Immunoglobulin Genes" pp. 124-149 Academic Press, London)).

25 As used herein the term "Ig fusion protein" means any recombinantly produced antigen or ligand binding domain having a constant region which can be structurally altered.

As used herein "cytotoxic agent" includes antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents. Specific examples within these groups include but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, 5 tenoposide, vincristine, vinblastine, colchicine, supporin, gelonin, PE40, bryodin, dihydroxy anthracin dione, actinomycin D, and 1-dehydrotestosterone.

As used herein the term "BR96" refers to (1) the whole BR96 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, (2) chimeric BR96 10 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, or (3) BR96 mutant molecules disclosed in PCT No. 95/305444, published March 6, 1996.

As used herein, "treating" means to (1) provide tumor regression so that the tumor is 15 not palpable for a period of time (standard tumor measurement procedures may be followed (A.B. Miller et al. "Reporting results of cancer treatment" Cancer 47:207-214 (1981)); (2) stabilize the disease; or (3) provide any clinically beneficial effects.

As used herein, an "effective amount" is an amount of the antibody, 20 immunoconjugate, or recombinant molecule which kills cells or inhibits the proliferation thereof.

As used herein, "administering" means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular or 25 subcutaneous administration, or the implantation of a slow-release device such as a miniosmotic pump, to the subject.

As used herein, "pharmaceutically acceptable carrier" includes any material which when combined with the antibody retains the antibody's specificity or efficacy and is

non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including
5 coated tablets and capsules.

Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may
10 also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods.

As used herein, "mutation" means a single amino acid or nucleic acid mutation or multiple mutations by whatever means, e.g., homologous recombination, error prone
15 PCR, or site directed mutagenesis.

In order that the invention herein described may be more fully understood, the following description is set forth.

20 METHODS OF THE PRESENT INVENTION

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from the use of immunoglobulin during therapy or in vivo diagnosis. For example, the methods of the invention would be useful to minimize
25 the toxicity associated with prolonged clinical exposure to immunoglobulin use during or after tumor imaging with radiolabeled antibodies.

In accordance with the practice of this invention, the subject includes, but is not limited to, human, equine, porcine, bovine, murine, canine, feline, and avian

subjects. Other warm blooded animals are also included in this invention.

This method comprises administering an immunoglobulin molecule to the subject. The immunoglobulin can be IgG, IgM, or IgA. IgG is preferred.

5

In one embodiment of the invention, the immunoglobulin molecule recognizes and binds Le^y. In another embodiment, the immunoglobulin recognizes and binds Le^x. In a further embodiment, the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma deposited on February 22, 1989 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and
10 accorded ATCC Accession No.: HB 10036. In yet another embodiment, the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma deposited on May 23, 1990, with the ATCC, 12301 Parklawn Drive, Rockville, MD 20852 and accorded ATCC Accession No.: HB 10460.

15

In accordance with the practice of the invention, the immunoglobulin can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the
20 ATCC binds. Also, in accordance with the practice of the invention, the immunoglobulin can be an anti-idiotypic antibody.

As required by the invention, at least a portion of the constant region of the immunoglobulin molecule is structurally altered. Structural alteration can be
25 effected by a number of means. In one embodiment, the entire constant region, i.e., CH₁, CH₂, and CH₃ domains, can be deleted.

In another embodiment, only the CH₂ domain is deleted from the immunoglobulin molecule (e.g., cBR96-A (Figure 5), hBR96-2A (Figure 4)). In this embodiment, the

CH₂ deletion may result in a molecule unable to bind the Fc receptor or a complement component.

In another embodiment, only that portion of the CH₂ domain which binds the complement component C1q is deleted. In yet another embodiment, mutations in specific portions of the CH₂ domain are made. For example, the immunoglobulin molecule may be modified by structurally altering multiple toxicity associated domains in the constant region so that immunoglobulin-induced toxicity is inhibited. A discussion of such mutations are further found hereinafter.

10

Regardless of the means, the underlying requirement for any structural alteration of the constant region is that immunoglobulin-induced toxicity is substantially reduced or inhibited. In one embodiment, immunoglobulin-induced toxicity is inhibited by structurally altering the constant region such that the molecule's ability to mediate a CDC response or ADCC response and/or activate the complement cascade is prevented or inhibited. Methods for determining whether the molecule is able to inhibit a CDC response are well known, e.g., one method involves a ⁵¹Cr-release test (H. Garrigues et al. Int. J. Cancer 29:511 (1982); I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to inhibit an ADCC response are well known (I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to activate a complement cascade are well known.

15

In another embodiment of the invention, the method comprises administering to the subject an Ig fusion protein having a structurally altered constant region. Structural alteration of the constant region may include deletion of the entire C region or portions thereof, e.g., alteration of the CH₂ domain so that the altered molecule no longer binds the Fc receptor or a complement component.

25

The invention further provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject. The method comprises administering to the subject an antibody which has been modified so that at least a portion of the constant region has been structurally altered as discussed supra. In one
5 embodiment, the antibody recognizes and binds Le^y . In another embodiment, the antibody recognizes and binds to Le^x .

In accordance with the practice of this invention, the antibody can be monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of
10 HB 10036 as deposited with the ATCC. Alternatively, the antibody can be chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC. Further, the antibody can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma
15 having the identifying characteristics of HB 10036 as deposited with the ATCC binds.

Additionally, the present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a
20 subject. The disease will vary with the antigen sought to be bound. Examples of diseases include but are not limited to immunological diseases, cancer, cardiovascular diseases, neurological diseases, dermatological diseases or kidney disease.

25 This method comprises the following steps. Step one provides selecting an antibody for a target. Generally, the target is associated with the disease and the antibody directed to the target is known. For example, the target can be the BR96 antigen and the antibody selected is BR96.

Step two of this method provides structurally altering the constant region of the antibody so selected so that immunoglobulin induced toxicity is inhibited. Inactivation can include any of the means discussed above. For example, inactivation can be effected by structurally altering multiple toxicity associated domains in the CH₂ domain of the constant region of the Ig protein so selected.

Step three of this method provides administering the structurally altered antibody of step two to the subject under conditions that the structurally altered antibody recognizes and binds the target and that such binding directly or indirectly alleviates symptoms associated with the disease.

In accordance with the invention, in one embodiment step one provides selecting an Ig fusion protein for a target. Further, the method provides mutating the Ig fusion protein so selected by structurally altering the CH₂ domain of the constant region of the Ig protein by the same means discussed above.

The invention further provides methods to treat human carcinoma. For example, the immunoglobulin, antibody, or Ig fusion protein discussed above can be used in combination with standard or conventional treatment methods such as chemotherapy, radiation therapy or can be conjugated or linked to a therapeutic drug, or toxin, as well as to a lymphokine or a tumor-inhibitory growth factor, for delivery of the therapeutic agent to the site of the carcinoma.

Techniques for conjugating therapeutic agents to immunoglobulins are well known (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellström et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In

Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)).

5

Alternatively, the structurally altered antibody or Ig fusion protein can be coupled to high-energy radiative agents, e.g., a radioisotope such as ^{131}I ; which, when localized at the tumor site, results in a killing of several cell diameters (see, e.g., Order, "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985)). According to yet another embodiment, the structurally altered BR96 antibody can be conjugated to a second antibody to form an antibody heteroconjugate for the treatment of tumor cells as described by Segal in United States Patent 4,676,980.

15

Still other therapeutic applications for the structurally altered antibody or Ig fusion protein of the invention include conjugation or linkage, e.g., by recombinant DNA techniques or protein chemical techniques, to an enzyme capable of converting a prodrug into a cytotoxic drug and the use of that antibody-enzyme conjugate in combination with the prodrug to convert the prodrug to a cytotoxic agent at the tumor site (see, e.g., Senter et al., "Anti-Tumor Effects Of Antibody-alkaline Phosphatase", Proc. Natl. Acad. Sci. USA, 85:4842-46 (1988); "Enhancement of the in vitro and in vivo Antitumor Activities of Phosphorylated Mitomycin C and Etoposide Derivatives by Monoclonal Antibody-Alkaline Phosphatase Conjugates", Cancer Research 49:5789-5792 (1989); and Senter, "Activation of Prodrugs by Antibody-Enzyme Conjugates: A New Approach to Cancer Therapy," FASEB J. 4:188-193 (1990)).

25

It is apparent therefore that the present invention encompasses pharmaceutical compositions including immunoglobulin molecules, antibodies, and Ig fusion proteins all having structurally altered CH₂ domains, and their use in methods for treating human carcinomas. For example, the invention includes pharmaceutical compositions for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of a structurally altered BR96 and a pharmaceutically acceptable carrier.

The compositions may contain the structurally altered antibody or Ig fusion protein or antibody fragments, either unmodified, conjugated to a therapeutic agent (e.g., drug, toxin, enzyme or second antibody). The compositions may additionally include other antibodies or conjugates for treating carcinomas (e.g., an antibody cocktail).

The compositions of the invention can be administered using conventional modes of administration including, but not limited to, intrathecal, intravenous, intraperitoneal, oral, intralymphatic or administration directly into the tumor. Intravenous administration is preferred.

The composition of the invention can be in a variety of dosage forms which include, but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

The compositions of the invention also preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate.

In accordance with the practice of the invention, the pharmaceutical carrier can be a lipid carrier. The lipid carrier can be a phospholipid. Further, the lipid carrier can be a fatty acid. Also, the lipid carrier can be a detergent. As used herein, a detergent
5 is any substance that alters the surface tension of a liquid, generally lowering it.

In one example of the invention, the detergent can be a nonionic detergent. Examples of nonionic detergents include, but are not limited to, polysorbate 80 (also known as Tween 80 or (polyoxyethylenesorbitan monooleate), Brij, and Triton (for
10 example Triton WR-1339 and Triton A-20).

Alternatively, the detergent can be an ionic detergent. An example of an ionic detergent includes, but is not limited to, alkyltrimethylammonium bromide.

15 Additionally, in accordance with the invention, the lipid carrier can be a liposome. As used in this application, a "liposome" is any membrane bound vesicle which contains any molecules of the invention or combinations thereof.

The most effective mode of administration and dosage regimen for the compositions
20 of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician.

The interrelationship of dosages for animals of various sizes and species and humans based on mg/m^2 of surface area is described by Freireich, E.J., et al. Cancer
25 Chemother., Rep. 50 (4): 219-244 (1966). Adjustments in the dosage regimen can be made to optimize the tumor cell growth inhibiting and killing response, e.g., doses can be divided and administered on a daily basis or the dose reduced proportionally depending upon the situation (e.g., several divided doses can be

administered daily or proportionally reduced depending on the specific therapeutic situation).

THE MOLECULES OF THE INVENTION

5

The present invention provides structurally altered BR96 or BR96 Ig fusion proteins.

Structurally altered BR96 antibodies or Ig fusion proteins have the variable region of BR96 and a modified constant region. This modification provides structurally altered BR96 antibodies or Ig fusion proteins with the ability to inhibit
10 immunoglobulin-induced toxicity.

Various embodiments of structurally altered BR96 or BR96 Ig fusion proteins have been made.

15 In one embodiment, designated cBR96-A, the entire CH₂ domain of cBR96 was deleted. CBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 10. cBR96 is expressed by a plasmid having the sequence in SEQ ID NO. 9.

20 In another embodiment, designated hBR96-2A, the entire CH₂ domain of hBR96 was deleted. hBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 12. hBR96 is a mutant BR96 having the H1, H2, and H3 mutations described in PCT Application No. 95/305444, published March 6, 1996.

25 In yet another embodiment, designated hBR96-2B, the leucine residue located at amino acid position 235 is mutated to alanine. Additionally, the glycine residue located at amino acid position 237 is mutated to alanine. The amino acid position numbering used is described in Kabat et al. Sequences of Proteins of Immunological Interest 5th Edition (1991) United States Department of Health and Human Services.

In a further embodiment, designated hBR96-2C, the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine using standard protocols (Alexander R. Duncan and Greg Winter "The binding site for C1q on IgG" Nature 332:738 (1988)).

In another embodiment, designated hBR96-2D, the proline residue at position 331 is mutated to alanine (M-H. Tao et al., "Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation" J. Exp. Med. 178:661-667 (1993); Y. Xu et al., "Residue at position 331 in the IgG1 and IgG4 domains contributes to their differential ability to bind and activate complement" J. Biol. Chem. 269:3469-3474 (1994)).

In an additional embodiment, designated hBR96-2E, the leucine residue at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine (A. Morgan et al., "The N-terminal end of the CH₂ domain of chimeric human IgG1 anti-HLA-DR is necessary for C1q, Fc(gamma)RI and Fc(gamma)RIII binding" Immunol. 86:319-324 (1995)).

In yet a further embodiment, designated hBR96-2F, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; and the proline residue located at position 331 is mutated to alanine.

In yet another embodiment, designated hBR96-2G, the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is

mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

5 In another embodiment, designated hBR96-2H, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

10

Depending on its form, a structurally altered BR96 antibody or fusion protein can be a monofunctional antibody, such as a monoclonal antibody, or bifunctional antibody, such as a bispecific antibody or a heteroantibody. The uses of structurally altered BR96, i.e., as a therapeutic or diagnostic agent, will determine the different forms of
15 structurally altered BR96 which is made.

Several options exists for antibody expression. Immunoexpression libraries can be combined with transfectoma technology, i.e., the genes for the Fab molecules derived from the immunoglobulin gene expression library can be connected to the
20 desired constant-domain exons. These recombinant genes can then be transfected and expressed in a transfectoma that would secrete an antibody molecule.

Once produced, the polypeptides of the invention can be modified, i.e., by amino acid modifications within the molecule, so as to produce derivative molecules. Such
25 derivative molecules would retain the functional property of the polypeptide, namely, the molecule having such substitutions will still permit the binding of the polypeptide to the BR96 antigen or portions thereof.

It is a well-established principle of protein chemistry that certain amino acid

substitutions, entitled "conservative amino acid substitutions," can frequently be made in a protein without altering either the conformation or the function of the protein.

- 5 Amino acid substitutions include, but are not necessarily limited to, amino acid substitutions known in the art as "conservative".

Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid
10 (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa.

Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional
15 structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine and valine (V).

Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R)
20 are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

- 25 In one embodiment of the present invention, the polypeptide is substantially pure, i.e., free of other amino acid residues which would inhibit or diminish binding of the polypeptide to its target and would inhibit or reduce gastrointestinal toxicity which are normally exhibited during or after antibody therapy.

NUCLEIC ACID MOLECULES ENCODING THE PRESENT INVENTION

The nucleotide sequences and the amino acid sequences of the variable and constant regions of BR96 are known. The sequence for the immunoglobulin constant region
5 is known and provided in Figure 18. Specific mutations in the constant region of the BR96 antibody were made. Nucleic acid molecules encoding the seven mutants described above (hBR96-2B through hBR96-2H) are as follows.

In hBR96-2B, alanine at amino acid positions 235 and 237 is encoded by codons
10 GCU, GCC, GCA, or GCG.

In hBR96-2C, serine at positions 318, 320, and 322 is encoded by UCU, UCC, UCA, or UGG.

15 In hBR96-2D, alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2E, alanine at positions 235 and 237 is encoded by codons GCU, GCC, GCA, or GCG. Serine at positions 318, 320, and 322 is encoded by UCU, UCC,
20 UCA, or UGG.

In hBR96-2F, alanine at positions 235, 237, and 331 is encoded by codons GCU, GCC, GCA, or GCG.

25 In hBR96-2G, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG. Further, the alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2H, alanine at positions 235, 237, and 331 is encoded by codons GCU,

GCC, GCA, or GCG. Additionally, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG.

Any of the above can be deoxyribonucleic acid (DNA), e.g., complementary DNA
5 (cDNA), or ribonucleic acid (RNA).

IMMUNOCONJUGATES

Immunoconjugates (having whole antibody or Ig fusion proteins) may be
10 constructed using a wide variety of chemotherapeutic agents such as folic acid and anthracyclines (Peterson et al., "Transport And Storage Of Anthracyclines In Experimental Systems And Human Leukemia", in Anthracycline Antibiotics In Cancer Therapy, Muggia et al. (Eds.), p. 132 (Martinus Nijhoff Publishers (1982); Smyth et al., "Specific Targeting of Chlorambucil to Tumors With the Use of
15 Monoclonal Antibodies", J. Natl. Cancer Inst., 76:503-510 (1986)), including doxorubicin (DOX) (Yang and Reisfeld "Doxorubicin Conjugated with a Monoclonal Antibody Directed to a Human Melanoma-Associated Proteoglycan Suppresses Growth of Established Tumor xenografts in Nude Mice PNAS (USA)" 85:1189-1193 (1988)), Daunomycin (Arnon and Sela "In Vitro and in vivo Efficacy
20 of Conjugates of Daunomycin With Anti-Tumor Antibodies" Immunol. Rev., 65:5-27 (1982)), and morpholinodoxorubicin (Mueller et al., "Antibody Conjugates With Morpholinodoxorubicin and Acid-Cleavable Linkers", Bioconjugate Chem., 1:325-330 (1990)).

25 BR96 has been conjugated to doxorubicin and has been shown to be effective in therapy of certain cancers or carcinomas (Trail, P.A., Willner, D., Lasch, S.J., Henderson, A.J., Casazza, A.M., Firestone, R.A., Hellström, I., and Hellström, K.E. Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. Science, 261:212-215, 1993).

In accordance with the practice of the invention, structurally altered BR96 can be used in forms including unreduced IgG, reduced structurally altered IgG, and fusion proteins (PCT Application No. 95/305444, published March 6, 1996).

5

Suitable therapeutic agents for use in making the immunoconjugate includes Pseudomonas exotoxin A (PE) in either the native PE or LysPE40 form. LysPE40 is a truncated form containing a genetically modified amino terminus that includes a lysine residue for conjugation purposes. Doxorubicin is also a suitable therapeutic agent.

10

Additional examples of therapeutic agents include, but are not limited to, antimetabolites, alkylating agents, anthracyclines, antibiotics, and anti-mitotic agents.

15

Antimetabolites include methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine.

Alkylating agents include mechlorethamine, thiotepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin.

20

Anthracyclines include daunorubicin (formerly daunomycin) and doxorubicin (also referred to herein as adriamycin). Additional examples include mitozantrone and bisantrene.

25

Antibiotics include dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC).

Antimitotic agents include vincristine and vinblastine (which are commonly referred to as vinca alkaloids).

- 5 Other cytotoxic agents include procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons.

- Further examples of cytotoxic agents include, but are not limited to, ricin, bryodin, gelonin, supporin, doxorubicin, taxol, cytochalasin B, gramicidin D, ethidium
10 bromide, etoposide, tenoposide, colchicine, dihydroxy anthracin dione, 1-dehydrotestosterone, and glucocorticoid.

- Clearly analogs and homologs of such therapeutic and cytotoxic agents are encompassed by the present invention. For example, the chemotherapeutic agent
15 aminopterin has a correlative improved analog namely methotrexate.

- Further, the improved analog of doxorubicin is an Fe-chelate. Also, the improved analog for 1-methylnitrosourea is lomustine. Further, the improved analog of vinblastine is vincristine. Also, the improved analog of mechlorethamine is
20 cyclophosphamide.

METHODS FOR MAKING MOLECULES OF THE INVENTION

- There are multiple approaches to making site specific mutations in the CH₂ domain
25 of an immunoglobulin molecule. One approach entails PCR amplification of the CH₂ domain with the mutations followed by homologous recombination of the mutated CH₂ into the vector containing the desired immunoglobulin, e.g., hBR96-2. For example, hBR96-2B and hBR96-2D have been made by this method.

Another approach would be to introduce mutations by site-directed mutagenesis of single-stranded DNA. For example, vector pD17-hG1b, which contains only the constant region of IgG1 and not the V domain of hBR96, has the fl origin of replication. This gives the vector the properties of a phagemid and site-directed mutagenesis experiments can be performed according to the methods of Kunkel, et al. (Kunkel, T.A., J.D. Roberts, and R.A. Zakour, 1987 Methods Enzymol. 154:367-383) as provided in the Bio-Rad Muta-Gene® phagemid *in vitro* mutagenesis kit, version 2. For example, hBR96-2B, -C, -D, -E, -F, -G, and -H were made by this method.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the scope of this invention in any manner.

EXAMPLE 1

The following standard ELISA protocol was used.

- Materials:** Immulon2 96 well plates and Genetic Systems Specimen Diluent Concentrate (10x); antibody conjugate was Goat Anti Human Kappa-HRP Mouse Adsorbed, Southern Biotech. at 1:10,000 in Genetic Systems Conjugate Diluent (1x); Genetic Systems EIA Chromogen Reagent (TMB) (1:100); Genetic Systems EIA Buffered Substrate (1x); primary antibody or antigen were AffiniPure F(ab')₂ Fragment Goat Anti Human IgG Fc Fragment specific (Jackson Immuno Research), Goat Anti Human Kappa-UNLB (Southern Biotechnology Associates), Le^y-HSA (Alberta Research Council).

Methods: Dilute primary antibody or antigen to 1.0 µg/ml in 0.05M Carb/Bicarb buffer. Add 100µl of the diluted solution per well in Immulon 2 plates. Seal plates and incubate O.N. at 4°C.

- 5 Block plates by flicking them and blotting on paper towels. Add 200µl/well of Genetic Systems, Specimen Diluent Concentrate (1x). Incubate at least 1 hour at room temperature and then dump the contents of the plates. Wash the plates 3x in saline/Tween. Blot to dry. Allow the plates to dry at R.T. (45 min. to 1 hour). Seal and store the plates at 4°C.

10

Test samples as follows. Dilute samples and standards in Specimen Diluent at 1:10. Perform serial dilutions in separate round bottom plates. Transfer 100µl/well of final dilutions to antigen coated assay plates; then incubate O.N. at 4°C. Wash plates 3x with saline/Tween.

15

For conjugation add 100 µl/well of antibody-HRP conjugate in Genetic Systems Conjugate Diluent (1x). Incubate plates at Room Temp. for 60 min. Wash plates 3x in saline/Tween.

- 20 Add 100 µl/well of Genetic Systems EIA Chromogen Reagent (TMB) 1:100 in EIA Buffered Substrate (1x). Incubate at R.T. for 15 min. and stop with 1N H₂SO₄ 100 µl/well. Read plate at 450/630nm in EIA plate reader.

EXAMPLE 2

25

Construction of CH₂ deleted BR96 molecules

Strategy for Deleting CH₂ Domains: To construct CH₂ deleted BR96 molecules, the hinge, CH₂ and CH₃ domains were removed from chimeric BR96 and humanized

BR9696-2 IgG1 molecules by an Eco47-III restriction digestion in non-coding regions. The hinge and CH₃ domains were amplified by polymerase chain reaction (PCR) from a human IgG1 (pNγ1.14) molecule lacking the CH₂ domain. Two oligonucleotides (Sense 49mer, Antisense 50mer) homologous to the sequences of
 5 IgG1 constant region at both sides preserving E.co47-III sites were synthesized. The amplified hinge and CH₃ domain PCR fragments were added into Eco47-III sites on BR96 IgG1 molecules by in vivo homologous recombination (P. Bubeck et al., Nucleic Acid Research (1993) 21:3601-3602). The new BR96 IgG1 molecules were verified by restriction mapping and sequencing.

10

A sewing PCR strategy was used for the construction of CH₂ deleted human IgG1 (pNγ1.14) (Robert M. Horton, et al. (1990) Biotech 8 (5)P, 528).

The CH₁ domain was amplified as a 580 bp fragment with a sense oligonucleotide
 15 (5' TGG CAC CGA **AAG CTT** TCT GGG GCA GGC CAG GCC TGA 3') (primer A) and an antisense oligonucleotide (5' **TCC GAG CAT GTT GGT ACC CAC GTG GTG GTC GAC** GCT GAG CCT GGC TTC GAG CAG ACA 3') (primer B) from a linearized human IgG1 constant region vector (pNγ1.7). The PCR fragment extends from the 5' end of the Hind-III site (in bold) through the Cel-II, Sal-I, Dra-
 20 III, Kpn-I, 6 bp nucleotide spacer and Mro-I sites (in bold) at the 3' end of the CH₁ domain.

The CH₃ domain was then partially amplified (to the Xba-I site) with a sense primer
 25 (5' **GTC GAC CAC CAC GTG GGT ACC AAC ATG TCC GGA GCC ACA TGG ACA GAG GCC GGC T** 3') (primer C) and an antisense primer (5' CTG GTT CTT GTT CAT CTC CTC **TCT AGA** TGG 3') (primer D) from a linearized human IgG1 constant region vector (pNγ1.7). A PCR fragment (about 150 bp) with Sal-I, Dra-III, Kpn-I, 6 nucleotide spacer and Mro-I sites (in bold) on its 5' end, extends only through the Xba-I site (in bold) within the CH₃ domain.

The CH₁ and CH₃ partial PCR fragments were combined in a PCR without any primer. The reaction was run through two full cycles of denaturation and re-annealing to allow the fragments to combine at the homologous region at the 3' ends. Primers A and D (described above) were added to the reaction and the PCR cycle was completed. The polymerase extends the DNA with primer A and primer D, yielding a full-length (660 bp) PCR fragment. The newly extended PCR fragment is arranged from the 5' end to the 3' end in the following order: Hind-III - CH₁ - Cel-II - Sal-I - Dra-III - Kpn-I - 6 bp spacer - Mro-I - CH₃ partial - Xba-I.

The combined PCR fragment, with the CH₁ and partial CH₃ domains, was then cloned by a blunt end ligation into a Sma-I site on a pEMBL18 vector and the sequence was confirmed by dideoxy sequencing (Sanger et al. (1977) PNAS (USA) 74:5463-5466).

To transfer the CH₁ and partial CH₃ into a mammalian expression vector, both the pEMBL18 and pNy1.7 vectors were digested with Hind-III and Xba-I. The Hind-III and Xba-I fragment was ligated into the same sites on a linearized pNy1.7 vector. The new construct, with CH₁ and a full CH₃ domain, was designated the pNy1.10 vector.

The hinge fragment was amplified from a Hind-III digested pNy1.7 vector with the primers designed to flank the hinge exon with a Sal-I and a Dra-III cloning site at each end. These sites also exist between the CH₁ and CH₃ domains of the pNy1.10 construct. The sense oligonucleotide (5' ACC ATG **GTC GAC** CTC AGA CCT GCC AAG AGC CAT ATC 3') with a 6 bp spacer and a Sal-I cloning site (in bold) and the antisense oligonucleotide (5' CAT GGT **CAC GTG** GTG TGT CCC TGG ATG CAG GCT ACT CTA G 3') with a 6 bp spacer and a Dra-III cloning site (in bold) were used for the amplification of the hinge fragment (250 bp).

The hinge region PCR fragment was cloned into a Sma-I site on pEMBL18 by blunt end ligation. Both the pEMBL18 with the hinge domain and the pN γ 1.10 with the CH₂ and CH₃ domains were digested with Sal-I and Dra-III. The digested hinge
5 fragment was cloned into the Sal-I and Dra-III linearized sites on the pN γ 1.10 vector. The new construct, now carrying the CH₁, hinge and CH₃ domains, was designated pN γ 1.11.

To make the final CH₂ deleted human IgG1 construct, both the pN γ 1.11 construct
10 and pN γ 1.11 vector were digested with BamHI and HindIII. A fragment containing the CH₁, hinge and CH₃ domains was cloned into the linearized pN γ 1.11 vector. The new constant region IgG1 construct lacks the CH₂ domain and is designated pN γ 1.14 (Figure 11).

15 For digestion of BR96 IgG1 with Eco47-III, a restriction fragment with hinge, CH₂ and CH₃ domains was identified on the constant region sequence of BR96 IgG1 vector in both chimeric and humanized molecules. The 5' end of this fragment lies inside the intron between CH₁ and hinge and the 3' end is located inside the CH₃ intron of the BR96 IgG1 molecule. The hinge, CH₂ and CH₃ domains (1.368 kb
20 fragment) were removed from BR96 IgG1 molecules by Eco47-III restriction digestion. The Eco47-III is a blunt end cutter. The BR96 IgG1 DNA digested with this enzyme does not require any pretreatment before cloning. Figure 12 is a diagrammatic representation of the pD17-hBR96-2 vector showing the Eco47-III sites used in cloning.

25

The CH₂ deleted BR96 IgG1 was then constructed as follows. The hinge and CH₃ domains were amplified from a CH₂ deleted L6 IgG1 (pN γ 1.14) construct with a sense oligonucleotide (5'
CAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCGCTGACCTCAG

- A 3') homologous to the constant region sequence of IgG1 at the 5' end of the Eco47-III site (in bold) and an antisense oligonucleotide (5'GGAAAGAACCATCACAGTCTCGCAGGGG CCCAGGGCAGCGCTGGGTGCTT 3') homologous to the constant region sequence of IgG1 at the 3' end of the Eco47-III site (in bold). The Eco47-III site at the 3' end of the pN γ 1.14 construct is modified in the cloning process. The Eco47-III site is thus introduced into an antisense primer and used in amplification of the hinge and CH₃ domains.
- 10 The pD17-BR96 IgG1 vector was digested with Eco47-III and the hinge, CH₂ and CH₃ domains were removed. The linearized pD17-BR96 IgG1 vector was mixed with equimolar amounts of hinge and CH₃ PCR fragments. Cotransformation of the PCR fragment with linearized DNA into E.coli DH5a competent cells resulted in a recombinant molecule, mediated by homologous recombination in bacteria. This
- 15 construct lacks the CH₂ domain of BR96 IgG1 molecules, and is designated pD17-BR96-dCH2 (Figure 13).

1.9 grams of CH₂-deleted chimeric BR96 was obtained as raw material from 89L of culture supernatant.

20

EXAMPLE 3

Toxicity, localization and clearance of CH₂-deleted chimeric BR96 was tested in vivo as follows.

25

Three dogs received 400 mg/m² of cBR96-A, the CH₂ deletion mutant of chimeric BR96, and two received chimeric BR96. Both molecules had been mildly reduced and alkylated. This is required to prevent dimerization of the deletion mutant into a tetravalent form. Both control dogs experienced the typical GI toxicity and none of

the three receiving the mutant displayed any toxicity. The control dogs and two of the test dogs were sacrificed at 1 hr to obtain duodenal tissue to measure antibody localization. Both control dogs had grossly visible GI pathology, and the test dogs had normal appearing GI tissue. The third dog has continued to show no signs of toxicity.

Results: A significant amount of localization of the CH₂ deleted cBR96 (cBR96-A) occurred to the GI tract in dogs treated with 400 mg/m², although the intact chiBR96 localized slightly better. The levels of localization indicate that roughly equivalent amounts of intact and CH₂ deleted cBR96 was delivered to the GI tract in these dogs.

Table 5. Localization of cBR96 to GI tissue.

Group	Animal	Specific	mean
		Localization	
cBR96	#271	155	135
	#272	114	
cBR96-A	#273	126	89
	#274	52	

15

Using the mean level of specific localization, an amount of cBR96-A equivalent to at least 66% of the amount of cBR96 was delivered to the target organ of toxicity, the duodenum. Based on the dose ranging done with cBR96 in dogs (some clinical signs of toxicity seen at doses of 10 mg/m²), even if this difference is real, it could

20

not explain the difference between significant toxicity and no toxicity, evaluation to date indicated that dogs treated with cBR96-A had no toxicity, pending microscopic histopathologic examination. This evaluation was based on analysis of 2 frozen blocks per dog and 2 sections per block. Replicates were quite good. We also ran
5 historical frozen tissues from dogs treated with native cBR96 or F(ab)₂/BR96 and the levels of localization for those tissues were 110 and 0, respectively, consistent with our previous data.

Assuming that there is no toxicity at marginally higher (2X) doses of cBR96-A,
10 these data indicate that the CH₂ domain is associated with the induction of acute gastroenteropathy, and that the removal of this domain prevents the induction of gastroenteropathy mediated by BR96.

This study confirms the results showing that F(ab')₂ is not toxic in the dog model
15 and that the toxicity is mediated by the constant region. The CH₂ deletion mutant is a candidate for targeting agents clinically. Because of the very long half-life of chimeric BR96, some decrease in the mutant's half-life should be acceptable.

Figure 1 shows the measurement of the clearance of the cBR96-A in high Le^y
20 expressing dogs. The study used chimeric versus constant region mutant of cBR96-2.

CBR96-2 did clear faster than the chimeric BR96. The localization of cBR96-A to the gastrointestinal epithelium is not significantly affected by this more rapid
25 clearance. More than enough of the cBR96-A localized to have caused toxicity.

Discussion: The constant region of chimeric IgG is responsible for the GI toxicity seen in clinical trials, e.g. with chiBR96-dox. The GI toxicity seen in the dog model is very similar to the clinical toxicity. Both in man and dog, administration of the

unconjugated antibody mediates an acute GI toxicity characterized by rapid onset of vomiting, often with blood.

5 In man the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach. Although the pathology of the wound is limited and resolves, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity.

10 This toxicity is mediated in man and dog by the antibody molecule alone. At higher doses of the antibody-dox conjugate, additional toxicity is seen in the dog model, probably due to doxorubicin. Although the intact IgG of BR96 causes toxicity in dog and man, the F(ab')₂ molecule (divalent and lacking only in the constant region) is not toxic in dogs. This finding has motivated our attempts at high levels, and improves the affinity and specificity of BR96 for tumor antigen.

15

The CH₂ domain is known to mediate complement and FcR binding. It was not known that structural alteration of the CH₂ domain would result in immunoglobulin-induced toxicity inhibition.

20 Toxicology study of hBR96-2B

The toxicology study of hBR96-2B in high Lewis Y expressor dogs (n=2) showed that a dose of 400 mg/m² did not cause hematemesis nor bloody stools, in contrast to BR96 which consistently causes one or both signs. A dog sacrificed at 24 hrs had
25 normal gross appearance of the GI tract, again in marked contrast to chimeric BR96 which causes hemorrhagic lesions and mucosal erosions.

EXAMPLE 4

- The polymerase chain reaction (PCR) is a widely used and versatile technique for the amplification and subsequent modification of immunoglobulin genes. The rapidity and accuracy with which antibody genes can be modified in vitro has produced an assortment of novel antibody genes can be modified in vitro has produced an assortment of novel antibodies. For example, PCR methods have been used for engineering antibodies with increased affinity to antigen, for "humanizing" antibodies, and for modulating effector function (Marks, J.D., A.D. Griffiths, M. Malmqvist, T. Clackson, J.M. Bye and G. Winter. 1992. Bypassing immunization: high affinity human antibodies by chain shuffling. *Bio/Technology* 10:779-783; Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96 Fab. *J. Biol. Chem.* 271:22611-22618; Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. *Immunology.* 86:319-324).
- As part of a more comprehensive study, we desired to introduce various site specific mutations in the CH₂ constant domain of human IgG₁. Six specific amino acid residues distributed throughout the CH₂ domain previously identified to play a role in immune effector function were marked as targets for mutagenesis (Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH₂ domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. *Immunology.* 86:319-324; Duncan, A.R. and G. Winter. 1988. The binding site for Clq on IgG. *Nature* 332:738-740; Tao, M.-H., R.I.F. Smith and S.L. Morrison. 1993. Structural features of human immunoglobulin G that determine isotype-specific differences in complement

activation. J.Exp.Med. 178:661-667). five of the six residues were grouped into two clusters-one cluster consisting of two residues, two amino acids apart (Location 1, or L1); and a second cluster consisting of three residues spanning a sequence of five amino acids (L2). The remaining amino acid position (L3) made for the total of six
5 residues. We were interested in constructing a panel of mutant CH₂ domain IgGs consisting of each L mutation by itself as well as in combination with other L mutants (e.g., L1; L1; and L2; L1, L2 and L3; etc.).

Various *in vitro* methods have been described where PCR is used to simultaneously
10 introduce distally located site-specific mutations within a gene sequence (Ho, S.N., H.D. Hunt, R.M. Horton, J.K. Pullen and L.R. Pease. 1989. Site-directed mutagenesis by overlap extension. Gene 77:51-59; Ge, L. and P. Rudolph. 1996. Simultaneous introduction of multiple mutations using overlap extension PCR. BioTechniques 22:28-30). Alternatively, an *in vivo* procedure termed recombination
15 PCR (RPCR) has also successfully been used for rapidly and efficiently generating distally located site-specific mutations (Jones, D.H. and S.C. Winistorfer. 1993. Use of polymerase chain reaction for making recombinant constructs. p.241-250. In B.A. White (Ed.), Methods in Molecular Biology, Vol. 15. Humana Press Inc., Totowa, NJ, Jones, D.H. And B.H. Howard. 1991. A rapid method for
20 recombination and site-specific mutagenesis by placing homologous ends on DNA using polymerase chain reaction. BioTechniques 10:62-66). RPCR uses *E. Coli*'s recombination machinery to generate intact circular recombinant plasmids from a transfected mixture of linear PCR-generated product and linearized vector. *In vivo* recombination is mediated through the joining of nucleotide sequences designed into
25 the 5' ends of both PCR primers that are homologous to DNA sequences encoded by the vector. In this report we describe an extension of the RPCR procedure for simultaneously introducing complex combinations of mutations into an antibody CH₂ domain.

- Humanized BR96 variable region heavy and light chain genes, previously cloned and co-expressed as an assembled active Fab fragment in an M13 phage expression vector, provided the starting material (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96 Fab. J. Biol. Chem. 271:22611-22618). The heavy and light chain V genes were amplified by PCR from a single-stranded M13 DNA template and subcloned by *in vivo* recombination (Jones, D.H. And B.H. Howard. 1991. A rapid method for recombination and site-specific mutagenesis by placing homologous ends on DNA using polymerase chain reaction. BioTechniques 10:62-66) into vectors pD17-hG1a and pD16-hCκ, to form pBR96-hG1a and pBR96-hCκ respectively. pD17-hG1a and pD16-hCκ are eukaryotic immunoglobulin expression vectors derived from pcDNA3 (Invitrogen, San Diego, CA). The plasmid pBR96-hG1a was further modified by site-directed mutagenesis to introduce two Eco47-III restriction sites flanking the immunoglobulin hinge-CH₂-CH₃ domains using standard procedures. The recipient vector was then prepared by digesting pBR96-hG1a with Eco47-III, isolating the vector backbone by agarose gel electrophoresis followed by extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA).
- The strategy for introducing multiple mutations within the immunoglobulin CH₂ gene, shown in Figure 24, relies on the *in vivo* homologous recombination of several independently amplified PCR products with each other as well as with the pBR96-hG1a vector DNA. For introducing mutations at two distal locations two PCR products are synthesized (Figure 24B). One end of each PCR product is for recombining with an homologous end of the linear vector, and the other end, encoding the mutation(s) of interest, is for recombining with the neighboring PCR product. As shown in Figure 24B, additional distally-located mutations can be introduced into a target sequence by increasing the number of PCR products

proportionately. The recombination of neighboring PCK' products always occurs across the regions containing the desired mutations. therefore the oligonucleotide primers encoding these ends (e.g., A1, A2) contain complementary mutant residues.

The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence
5 flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers (Rs and Ra) contain sequences for recombining with the end regions of the Eco47-III digested pBR96-hG1a vector.

10 Each L mutation was amplified in a separate PCR reaction. The reaction conditions were 250 ng intact pBR96-hG1a DNA template, 10 ul of 1X *Pfu* buffer (Stratagene, Inc. San Diego, CA), 10 nmol dNTPs, 200ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase in a 100ul reaction volume. Samples were first denatured at 95° C for 5
15 min, cooled to 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, followed by a final extension at 72°C for 7 min in a Perkin-Elmer DNA Thermal Cycler (Norwalk, CT). The amplified products were purified from a 1% agarose gel, extracted with Qiagen Gel Extraction kit and the recovered
20 DNA quantitated. 50 ng of each PCR product was mixed with 25 ng of the Eco47-III digested pBR96-hG1a vector, transfected into Max competent *E. coli* DH5α according to the manufacturer's procedure (GIBCO BRL/Life Technologies, Gaithersburg, MD), and the entire transfection reaction plated onto selective LB agar plates containing 100 ug/ml ampicillin.

25

The results of several cloning experiments are summarized in the Table that follows. Typically the transformations produced from 80 to 200 bacterial colonies. Individual colonies were selected and grown overnight in 2 ml liquid cultures for isolation of miniprep plasmid DNA (Qiagen) and analysis by Eco47-III restriction

endonuclease mapping. Among 24 independent transformants analyzed from triple homologous recombination events (two PCR products plus vector) 11 clones contained the predicted 1.4 kpb DNA insert.

- 5 Figure 25 shows a sample diagnostic restriction analysis of DNA prepared from clones derived from quadruple homologous recombination events (three PCR products plus vector). Additional sampling of clones resulting from quadruple recombination yielded a cloning efficiency of 29% (7 clones containing inserts/24 clones sampled). At this point, due to the small sampling sizes, we do not know
10 whether the differences in the cloning efficiencies observed between the triple and quadruple recombination events are meaningful.

- To evaluate the expression of Le γ -binding activity of the CH₂ mutant IgGs, miniprep DNAs from 6 clones derived from the triple recombination reaction and 6
15 clones derived from the quadruple recombination reaction exhibiting the predicted diagnostic Eco47-III restriction patterns were isolated, mixed with pBR96- hC κ DNA and used to co-transfect COS7 cells. 48 hour spent supernatants from 3 ml cultures were assayed for total IgG production and for Le γ binding activity by enzyme-linked immunosorbent assay (EIA) as described (Yelton, D.E., M.J. Rosok,
20 G.A. Cruz, W.L. Cosand, J. Bajorath, I. Hellstom, K.-E. Hellstorm, W.D. Huse and S.M. Glaser. 1995. Affinity maturation of the BR96 anti-carcinoma antibody by codon-based mutagenesis. J.Immunol. 155:1994-2004). All twelve cultures were found to secrete approximately 2-3 ug/ml Le γ -reactive IgG. The spectrum of Le γ binding activities were all similar to that of native humanized BR96 IgG indicating
25 that the homologously recombined antibodies did not acquire any gross mutations that could affect antigen binding. To confirm that the desired CH₂ mutations had been incorporated, and to evaluate the recombined genes for misincorporated nucleotides, four of the clones producing functional antibody were sequenced using Sequenase Version 2 DNA Sequencing Kit (United States Biochemical). One clone

was found to contain a single nucleotide change within the forward PCR primer used for mediating recombination with vector DNA. We are uncertain whether this error occurred during chemical synthesis of the oligonucleotide primer or is a result of misincorporation during the PCR reaction, despite the fact that we used a

5 thermostable polymerase with proofreading activity.

A RPCR procedure for homologously recombining up to three separate PCR-generated mutated antibody sequence products into a eukaryotic expression vector for the rapid construction of engineered IgG molecules is described herein. The

10 advantage of this approach is the ability to simultaneously introduce multiple distally-located mutations with PCR products synthesized by a single round of PCR. Recombinant DNAs are produced with a reasonably high cloning efficiency and fidelity of correct nucleotide sequences. The ability to efficiently rejoin several distinct PCR products should permit combinatorial strategies for constructing

15 complexly mutated protein domains as well as broadening the number and location of desired mutations.

Analysis of transformants generated by multiple-fragment RPCR.

Mutant IgGs Constructed	PCR Fragments in reaction	HR ^a events	Colonies Analyzed	Cloning Efficiency ^b
2	2	triple	24	45%
2	3	quadruple	24	33%
^a HR-homologous recombination				
^b Cloning efficiency (number of clones containing 1.4kbp insert/total number of colonies)				

EXAMPLE 5

This example provides two methods for introducing site specific mutations into the
5 CH2 domain of human IgG1 constant region containing vectors.

One method involves PCR amplification of a segment or segments of the constant region, wherein mutations are introduced using appropriately constructed oligonucleotides. The vector receiving the fragment(s) is digested with a restriction
10 enzyme to linearize the vector. PCR amplification primers are designed so that the 5' ends of the PCR fragments can hybridize to the DNA sequence of the vectors. If more than one PCR fragment is amplified, then common sequences to the two fragments are introduced by oligonucleotides. Bacteria are transfected with the PCR fragments and with the digested vector. The fragments and vector can recombine by
15 homologous recombination using the bacteria's recombination machinery. Bacterial colonies are selected and the DNA is analyzed by size and restriction map as a preliminary determination that the vector and fragment(s) recombined correctly. Correct insertion of fragments with the mutations is confirmed by dideoxynucleotide sequence analysis. DNA is then introduced into mammalian cells as described for
20 the CH2 deleted antibody, and the expressed antibody analyzed for binding and functional activity.

By way of example, mutations Leu to Ala at residue 235 in CH2 and Gly to Ala at residue 237 were introduced by the procedure disclosed in Example 4. The heavy
25 chain vector used for this procedure was pD17-hG1a, similar to pD17-BR96 vector described herein except that humanized V regions (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse, and S.M. Glaser, 1996. J. Biol. Chem 271 37:22611-22618) with three affinity mutations (H1, H2, and H3 mutations) were substituted.

pBR96-hG1a contains two *Eco47-III* restriction sites flanking the Ig hinge-CH2-CH3 domains. The recipient vector was prepared by (1) digesting pBR96-hG1a with *Eco47-III*, (2) isolating the vector by agarose gel electrophoresis, and (3)
5 extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA). To introduce mutations at a single location, such as for positions 235 and 237, two PCR products were synthesized.

To introduce two distally located mutations, such as for mutant F (also referred to
10 herein as hBR96-2F) with mutations at 235, 237, 331, requires 3 PCR products. The recombination of neighboring PCR products occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends contain complementary mutant residues. The mutagenic PCR primers contain at least 15
15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers containing sequences for recombining with the end regions of the *Eco47-III* digested pBR96-hG1a vector.

PCR amplification used 250 ng intact pBR96-hG1a DNA template, 10 μ l of 10X *Pfu*
20 buffer (Stratagene, Inc., San Diego, CA), 10 nmol dNTPs, 200 ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase (Stratagen, Inc. San Diego, CA) in 100 μ l reaction. Samples were denatured at 95°C for 5 min, annealed at 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45
25 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, and a final extension at 72°C for 7 min. The amplified products were purified from a 1% agarose gel, extracted with the Qiagen Gel Extraction kit and quantitated. 50 mg of each PCR product was mixed with 25 ng of the *Eco47-III* digested pBR96-hG1a vector and transfected in E.coli MAX Efficiency DH5 α TM according to the

manufacturer's instructions (GIBCO BRL/Life Technologies, Gaithersburg, MD).

The entire transfection reaction was plated onto LB agar plated containing 100 µg/ml ampicillin.

- 5 Bacterial colonies were selected and grown overnight at 37° C in 2 ml liquid cultures. DNA was isolated and analyzed by Eco47-III restriction endonuclease mapping. Clones with the correct size insert were sequenced (Sequenase Version 2, U.S. Biochemical Corp., Cleveland, OH).
- 10 The second method for introducing site specific mutations into the CH₂ domain of human IgG1 involved the method of Kunkel (1987 Methods Enzymology, supra). For this procedure pD17-hG1b DNA with the F1 origin of replication was introduced into electrocompetent E. coli CJ236 dut-ung- (Bio-Rad Laboratories, Hercules, CA) by electroporation according to manufacturer's instructions. PD17-
15 hG1b is a vector having a constant region but no variable region. The F1 ori site allows treatment of this vector as a phagemid.

Bacteria containing the plasmid were selected by ampicillin resistance. Single stranded uridynylated DNA was prepared using the Muta-Gene Phagemid In Vitro

- 20 Mutagenesis Version 2 protocol (Bio-Rad). Mutations were introduced by site-directed mutagenesis with the appropriate antisense oligonucleotide. For molecules with mutations at more than one location, mutations were introduced by either of the two methods discussed above. One method would be to (1) prepare one mutant, for example, mutant 2C (also referred to herein as BR96-2C) with the mutations at
25 residues 318, 320, 322, (2) isolate ssDNA, and (3) introduce a second mutation set with the appropriate anti-sense oligonucleotide. The second method would be to anneal two antisense oligonucleotides with the same uridynylated ssDNA and screen for mutants with both sets of changes. Mutant 2H (hBR96-2H) was also prepared by a combination of these methods.

The V region of humanized BR96-2 heavy chain was introduced by the homologous recombination method described above in pD17-hJm14.H1. The pD17-hJm14.H1 plasmid contains the BR96 humanized variable region with the H1/H2/H3

- 5 mutations and the plasmid was used to transfect mutant sequences into mammalian cells. The pD17G1b vector containing the Fc mutation(s) was digested with NheI for 3 hr at 37° C and the DNA isolated by methods described above. Insertion of the V region into the vector was determined by size and restriction enzyme mapping and confirmed by sequence analysis.

10

Transient expression of whole antibodies was performed by transfection of COS cells. For production of antibody, stable transfections of CHO cells were performed (see description of deleted CH2 mutant). All mutants were purified from CHO culture supernatants by protein A chromatography.

15

The oligonucleotide primers homologous to the vector and used to introduce the constant regions mutations were as follows:

Oligonucleotides homologous to vector sequences:

Sens(sense)CH2 E47-3-5: CAG GGA GGG AGG GTG TCT GCT GGA AGC

- 20 CAG GCT CAG CGC TGA CCT CAGA

D CH2 E47-3 A (antisense): GGA AAG AAC CAT CAC AGT CTC GCA GGG
GCC CAG GGC AGC GCT GGG TGC TT

Oligonucleotides to mutate Leu235 to Ala and Gly237 to Ala (underlined sequences

- 25 show sites of mutation):

Antisense CH2 L235-G237/aa: GAA GAG GAA GAC TGA CGG TGC CCC
CGC GAG TTC AGG TGC TGA GG

SensCH2 L235-G237/AA: CCT CAG CAC CTG AAC TCG CGG GGG CAC
CGT CAG TCT TCC TCT TC

Oligonucleotides to mutate Glu318, Lys320, Lys322 to Ser

Antis(antisense)CH2 EKK/SSS-2: CTG GGA GGG CTT TGT TGG AGA CCG
AGC ACG AGT ACG ACT TGC CAT TCA GCC

5 Oligonucleotides to mutate Pro331 to Ala:

Antis CH2 P331/A/3: GAT GGT TTT CTC GAT GGC GGC TGG GAG GGC

Sense CH2 P33/A: GCC CTC CCA GCC GCC ATC GAG AAA ACC ATC

Alternative antisense oligo to introduce Ala at 331 by site-directed mutation:

CH2P331A: GAT GGT TTT CTC GAT AGC GGC TGG GAG GGC TTT G

10

Oligonucleotides to mutate Glu318 to Ser, Lys320 to Ser, Lys322 to Ser, and Pro331 to Ala:

Antis CH2 EKKP/SSA-6: GAT GGT TTT CTC GAT GGC GGC TGG GAG
 GGC TTT GTT GGA GAC CGA GCA CGA GTA CGA CTT GCC ATT CAG

15 CCA GTC CTG GTG

Sense CH2 EKKP/SSA-6: CAC CAG GAC TGG CTG AAT GGC AAG TCG
 TAC TCG TGC TCG GTC TCC AAC AAA GCC CTC CCA GCC GCC ATC
 GAG AAA ACC ATC

20

In vitro Assays of the Mutants

Results of the CDC demonstrate that mutant hBR96-2B has approximately 10 fold less activity than the control hBR96-1 (two affinity mutations, one in H2 and one in H3, refer to previous patent (Figure 20)). The mutants that have the least ability to kill cells in the presence of complement is hBR96-2C with the triple mutations at positions 318, 320, and 322 and the hBR96-2H mutant (least cytotoxic antibodies in the panel) which contains all six mutations at the three different locations. ADCC activity was most affected by the CH2 deleted hBR96-2 molecule (Figure 21).

hBR96-2B and -2H lost between 100 and 1000 fold activity to kill in the presence of effector cells. In the ADCC assay the hBR96-2B molecule also lost approximately 10 fold activity (Figure 21).

- 5 Figures 26-28 provide the amino acid sequences for the heavy chain variable region for both chimeric and humanized BR96 having the H1, H2, and H3 mutations. The amino acid sequence for the light chain variable region is known and methods for generating it are found in PCT Application No. 95/305444. Additionally provided is the amino acid sequence for the IgG1 constant region. Mutations in the constant
- 10 region are marked.

SEQUENCE LISTING

- 5 (1) GENERAL INFORMATION
- (i) APPLICANT: Bristol-Myers Squibb Co.
- 10 (ii) TITLE OF THE INVENTION:
A METHOD FOR INHIBITING
IMMUNOGLOBULIN-INDUCED TOXICITY FROM THE USE OF
IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS
- (iii) NUMBER OF SEQUENCES: 13
- 15 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Merchant & Gould
(B) STREET: 11150 Santa Monica Blvd., Suite 400
(C) CITY: Los Angeles
(D) STATE: CA
20 (E) COUNTRY: USA
(F) ZIP: 90025
- (v) COMPUTER READABLE FORM:
25 (A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 2.0
- 30 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US97/_____
(B) FILING DATE: 01-AUG-1997
(C) CLASSIFICATION:
- 35 (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/023,033
(B) FILING DATE: 02-AUG-1996
- 40 (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Adriano, Sarah B
(B) REGISTRATION NUMBER: 34,470
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- 45 (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 310-445-1140
(B) TELEFAX: 310-445-9031
(C) TELEX:
- 50 (2) INFORMATION FOR SEQ ID NO:1:
- 55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5 TGGCACCGAA AGCTTTCTGG GGCAGGCCAG GCCTGA 36

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

20 TCCGGACATG TTGGTACCCA CGTGGTGGTC GACGCTGAGC CTGGCTTCGA GCAGACA 57

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

35 GTCGACCACC ACGTGGGTAC CAACATGTCC GGAGCCACAT GGACAGAGGC CGGCT 55

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTGGTTCTTG TTCATCTCCT CTCTAGATGG 30

(2) INFORMATION FOR SEQ ID NO:5:

50 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
55 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACCATGGTCG ACCTCAGACC TGCCAAGAGC CATATC 36

(2) INFORMATION FOR SEQ ID NO:6:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

15 CATGGTCACG TGGTGTGTCC CTGGATGCAG GCTACTCTA 39

(2) INFORMATION FOR SEQ ID NO:7:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 49 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

30 CAGGGAGGGA GGGTGTCTGC TGGAAGCCAG GCTCAGCGCT GACCTCAGA 49

(2) INFORMATION FOR SEQ ID NO:8:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 50 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

45 GGAAAGAACC ATCACAGTCT CGCAGGGGCC CAGGGCAGCG CTGGGTGCTT 50

(2) INFORMATION FOR SEQ ID NO:9:

50 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8691 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCCG GCTTCGAATA GCCAGAGTAA 60

CCTTTTTTTT TAATTTTATT TTATTTTATT TTTGAGATGG AGTTTGGCGC CGATCTCCCG 120

	ATCCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGC	GAGCAAAATT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGTTTTGCGC	300
	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAAT	360
5	AGTAATCAAT	TACGGGGTCA	TTAGTTTATA	GCCCATATAT	GGAGTTCCGC	GTTACATAAC	420
	TTACGGTAAA	TGGCCCGCCT	GGCTGACCGC	CAAACGACCC	CCGCCCATTG	ACGTCAATAA	480
	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTTACGGTA	AACTGCCCCA	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCC	CCTGGCATTG	TGCCCAGTAC	ATGACCTTAT	660
10	GGGACTTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	720
	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCAC	TGCTTACTGG	CTTATCGAAA	960
15	TTAATACGAC	TCACTATAGG	GAGACCCAAG	CTTGGTACCA	ATTAAATTG	ATATCTCCTT	1020
	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGCGGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCCT	TGTTTTAAAA	GGTGTCCAGT	1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
	TCTCCTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATTA	CATGTATTGG	GTTTCGCCAGA	1260
20	TCCCAGAGAA	GAGGCTGGAG	TGGGTGCGAT	ACATTAGTCA	AGGTGGTGAT	ATAACCGACT	1320
	ATCCAGACAC	TGTAAAGGGT	CGATTACCA	TCTCCAGAGA	CAATGCCAAG	AACACCCTGT	1380
	ACCTGCAAT	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500
	CTAGCACCAA	GGGCCCATCG	GTCTTCCCCC	TGGCACCCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
25	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCCTGT	ACGGTGTCTG	1620
	GGAACTCAGG	CGCCCTGACC	AGCGCGTGC	ACACTTCCC	GGCTGTCTTA	CAGTCTCTAG	1680
	GACTCTACTC	CCTCAGCAGC	GTGGTCAACG	TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCT	1740
	ACATCTGCAA	CGTGAATCAC	AAGCCAGCA	ACACCAAGGT	GGACAAGAAA	GTTGGTGAGA	1800
	GGCCAGCACA	GGGAGGGAGG	GTGTCTGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCTGGACG	1860
30	CATCCCGGCT	ATGCAGCCCC	AGTCCAGGGC	AGCAAGGCAG	GCCCCGTCTG	CCTCTTACC	1920
	CGGAGGCCTC	TGCCCCCCCC	ACTCATGCTC	AGGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG	1980
	GCTCTGGGCA	GGCACAGGCT	AGGTGCCCTC	AACCCAGGCC	CTGCACACAA	AGGGGCAGGT	2040
	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC	CGGGAGGACC	CTGCCCTGA	CCTAAGCCCA	2100
	CCCCAAAGGC	CAACTCTCTC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA	2160
35	GTAACCTCCA	ATCTTCTCTC	TGCAGAGCCC	AAATCTTGTG	ACAAAACCTCA	CACATGCCCA	2220
	CCGTGCCCCG	GTAAGCCAGC	CCAGGCCTCG	CCCTCCAGCT	CAAGGCGGGA	CAGGTGCCCT	2280
	AGAGTAGCCT	GCATCCAGGG	ACAGGCCCCA	GCCGGGTGCT	GACACGTCCA	CCTCCATCTC	2340
	TTCTCTAGCA	CCTGAACTCC	TGGGGGGACC	GTCAGTCTTC	CTCTTCCCCC	CAAAACCCAA	2400
	GGACACCCTC	ATGATCTCCC	TGACCCCTGA	GGTCACATGC	GTGGTGGTGG	ACGTGAGCCA	2460
40	CGAAGACCTT	GAGGTCAAGT	TCAACTGGTA	CGTGACGGC	GTGGAGGTGC	ATAATGCCAA	2520
	GACAAAGCCG	CGGGAGGAGC	AGTACAACAG	CACGTACCGT	GTGGTCAGCG	TCCTCACCGT	2580
	CCTGCACCAG	GACTGGCTGA	ATGGCAAGGA	GTACAAGTGC	AAGGTCTCCA	ACAAAGCCCT	2640
	CCCAGCCCCC	ATCGAGAAAA	CCATCTCCAA	AGCCAAAGGT	GGGACCCGTG	GGGTGCGAGG	2700
	GCCACATGGA	CAGAGGCCGG	CTCGGCCAC	CCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	2760
45	CCTCTGTCCC	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA	CACCCTGCCC	CCATCCCGGG	2820
	ATGAGCTGAC	CAAGAACCAG	GTCAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCCAGCG	2880
	ACATCGCCGT	GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG	ACCACGCCTC	2940
	CCGTGCTGGA	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA	GCTCACCGTG	GACAAGAGCA	3000
	GGTGGCAGCA	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	3060
50	ACACGCAGAA	GAGCCTCTCC	CTGTCTCCGG	GTAATGAGT	GCGACGGCCG	GCAAGCCCCC	3120
	GCTCCCCGGG	CTCTCGCGGT	CGCACAGGGA	TGCTTGGCAC	GTACCCCTG	TACATACTTC	3180
	CCGGGGCGCC	AGCATGGAAA	TAAAGCACCC	AGCGCTGCCC	TGGGCCCCCTG	CGAGACTGTG	3240
	ATGGTTCTTT	CCACGGGTCA	GGCCGAGTCT	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	3300
	GGGTCCCACT	GTCCCCACAC	TGGCCCAGGC	TGTGCAAGTG	TGCCTGGGCC	CCCTAGGGTG	3360
55	GGGCTCAGCC	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	3420
	AGCAGCACCT	GCCCTGGGCT	GGGCCACCGG	AAGCCCTAGG	AGCCCTGGG	GACAGACACA	3480
	CAGCCCCCTG	CTCTGTAGGA	GACTGTCTGG	TTCTGTGAGC	GCCCTGTCTC	TCCCGACCTC	3540
	CATGCCCACT	CGGGGGCATG	CCTAGTCCAT	GTGCGTAGGG	ACAGGCCCTC	CCTCACCCAT	3600
	CTACCCCCAC	GGCACTAACC	CCTGGCTGCC	CTGCCCAGCC	TCGCACCCGC	ATGGGGACAC	3660

	AACCGACTCC	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GA CTG ⁵ GTGCA	GATGCCCACA	3720
	CACACACTCA	GCCCAGACCC	GTTCAACAAA	CCCCGCACTG	AGGTTGGCCG	GCCACACGGC	3780
	CACCACACAC	ACACGTGCAC	GCCTCACACA	CGGAGCCTCA	CCCGGGCGAA	CTGCACAGCA	3840
	CCCAGACCAG	AGCAAGGTCC	TCGCACACGT	GAACACTCCT	CGGACACAGG	CCCCCACCAG	3900
5	CCCCACGCGG	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	3960
	TCAGACAAAC	CCAGCCCTCC	TCTCACAAGG	GTGCCCTGTC	AGCCGCCACA	CACACACAGG	4020
	GGATCACACA	CCACGTCACG	TCCCTGGCCC	TGGCCCACTT	CCCAGTGCCG	CCCTTCCCTG	4080
	CAGGACGGAT	CAGCCTCGAC	TGTGCCTTCT	AGTTGCCAGC	CATCTGTTGT	TTGCCCTCC	4140
	CCCGTGCCCT	CCTTGACCCT	GGAAGGTGCC	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	4200
10	GAAATTGCAT	CGCATTGTCT	GAGTAGGTGT	CATTCTATTG	TGGGGGGTGG	GGTGGGGCAG	4260
	GACAGCAAGG	GGGAGGATTG	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	4320
	ATGGCTTCTG	AGGCGGAAAG	AACCAGCTGG	GGCTCTAGGG	GGTATCCCCA	CGCGCCCTGT	4380
	AGCGGCGCAT	TAAGCGCGGC	GGGTGTGGTG	GTTACGCGCA	GCGTGACCGC	TACACTTGCC	4440
	AGCGCCCTAG	CGCCGCTCC	TTCGCTTTG	TTCCCTTCCT	TTCTCGCCAC	GTTGCGCCGG	4500
15	CCTCTCAAAA	AAGGGAAAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCC GCCC	4560
	CTAACTCCGC	CCATCCCGCC	CCTAACTCCG	CCCAGTTCCG	CCCATTCTCC	GCCCCATGGC	4620
	TGACTAATTT	TTTTTATTTA	TGCAGAGGCC	GAGGCCGCCT	CGGCCTCTGA	GCTATTCCAG	4680
	AAGTAGTGAG	GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	4740
	GCTGCGATT	CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	4800
20	CCCGCTGCCA	TCATGTTTCG	ACCATTGAAC	TGCATCGTCG	CCGTGTCCCA	AAATATGGGG	4860
	ATTGGCAAGA	ACGGAGACCT	ACCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTA CTTC	4920
	AGAATGACCA	CAACCTCTTC	AGTGAAGGT	AAACAGAATC	TGGTGATTAT	GGGTAGGAAA	4980
	ACCTGGTTCT	CCATTCTCTG	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	5040
	AGTAGAGAAC	TCAAAGAACC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG	TTTGGATGAT	5100
25	GCCTTAAGAC	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	5160
	GGAGGCAGTT	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	5220
	ACAAGGATCA	TGCAAGGAATT	TGAAAGTGAC	ACGTTTTTCC	CAGAAATTGA	TTTGGGGAAA	5280
	TATAAACTTC	TCCCAGAATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	5340
	AAGTATAAGT	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	5400
30	GCTCCCCTCC	TAAAGCTATG	CATTTTATA	AGACCATGGG	ACTTTTGCTG	GCTTTAGATC	5460
	TCTTTGTGAA	GGAACTTAC	TTCTGTGGTG	TGACATAATT	GGACAACTA	CCTACAGAGA	5520
	TTTAAAGCTC	TAAGGTAAAT	ATAAAATTTT	TAAGTGTATA	ATGTGTTAAA	CTACTGATTC	5580
	TAATTGTTTG	TGTATTTTAG	ATTCCAACCT	ATGGAAGTGA	TGAATGGGAG	CAGTGGTGGA	5640
	ATGCCTTTAA	TGAGGAAAAC	CTGTTTGTCT	CAGAAGAAAT	GCCATCTAGT	GATGATGAGG	5700
35	CTACTGCTGA	CTCTCAACAT	TCTACTCCTC	CAAAAAGAGAA	GAGAAAGGTA	GAAGACCCCA	5760
	AGGACTTTCC	TTCAAGATTG	CTAAGTTTTT	TGAGTCATGC	TGTGTTTAGT	AATAGAACTC	5820
	TTGCTTGCTT	TGCTATTTAC	ACCACAAAGG	AAAAGCTGTC	ACTGCTATAC	AAGAAAATTA	5880
	TGGAAAAATA	TTCTGTAAAC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	5940
	TTTTTCTTAC	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACATGCT	CAAAAATTGT	6000
40	GTACCTTTAG	CTTTTTTAAT	TGTAAAGGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCCCT	6060
	TGACTAGAGA	TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTTACTTGC	TTTAAAAAAC	6120
	CTCCCACACC	TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	6180
	TTTATTGCAG	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	6240
	GCATTTTTTT	CACTGCATTG	TAGTTGTGGT	TGTGCCAAAC	TCATCAATGT	ATCTTATCAT	6300
45	GTCTGGATCG	GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCCCAC	6360
	CCCAACTTGT	TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTT	6420
	ACAAATAAAG	CATTTTTTTT	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	6480
	TCTTATCATG	TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	6540
	CTGTTTCTTG	TGTGAAATTG	TTATCCGCTC	ACAATTCCAC	ACAACATACG	AGCCGGAAGC	6600
50	ATAAAGTGTA	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAAT	TGCGTTGCGC	6660
	TCACTGCCCC	CTTCCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAAAT	AATCGGCCAA	6720
	CGCGCGGGGA	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCCG	CTTCCTCGCT	CACTGACTCG	6780
	CTGCGCTCGG	TCGTTCCGGT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	6840
	TTATCCACAG	AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	6900
55	GCCAGGAACC	GTA AAAAGGC	CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	6960
	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGCGGAA	ACCCGACAGG	ACTATAAAGA	7020
	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	7080
	ACCGGATACC	TGTCGCGCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTCTCA	ATGCTCACGC	7140
	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	7200

	CCCGTTCAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	7260
	AGACACGACT	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	7320
	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	7380
	GTATTTGGTA	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	7440
5	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	7500
	ACGCGCAGAA	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	7560
	CAGTGGAACG	AAAACCTACG	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	7620
	ACCTAGATCC	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	7680
	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	7740
10	TTTCGTTTCAT	CCATAGTTGC	CTGACTCCCC	GTCGTGTAGA	TAACTACGAT	ACGGGAGGGC	7800
	TTACCATCTG	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	7860
	TTATCAGCAA	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	7920
	TCCGCCTCCA	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCCGACGTT	7980
	AATAGTTTGC	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACAG	CTCGTCGTTT	8040
15	GGTATGGCTT	CATTGAGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	8100
	TTGTGCAAAA	AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTCAGAAG	TAAGTTGGCC	8160
	GCAGTGTTAT	CACTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	8220
	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	8280
	CGGCGACCGA	GTTGCTCTTG	CCCGGCGTGA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	8340
20	ACTTTAAAAA	TGCTCATCAT	TGGAAAACGT	TCTTCGGGCG	GAAAACTCTC	AAGGATCTTA	8400
	CCGCTGTTGA	GATCCAGTTC	GATGTAACCC	ACTCGTGAC	CCAACTGATC	TTCAGCATCT	8460
	TTTACTTTCA	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAAG	8520
	GGAATAAGGG	CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTTCA	ATATTATTGA	8580
	AGCATTATATC	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	8640
25	AAACAAATAG	GGGTTCGCG	CACATTTCCC	CGAAAAGTGC	CACCTGACGT	C	8691

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 8327 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

	GACGGATCGG	GAGATCTGCT	AGGTGACCTG	AGGCGCGCCG	GCTTCGAATA	GCCAGAGTAA	60
40	CCTTTTTTTT	TAATTTTATT	TTATTTTATT	TTTGAGATGG	AGTTTGGCGC	CGATCTCCCG	120
	ATCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGC GC	GAGCAAAATT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGTTTTGCGC	300
	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAAT	360
45	AGTAATCAAT	TACGGGGTCA	TTAGTTCATA	GCCCATATAT	GGAGTTCCGC	GTTACATAAC	420
	TTACGGTAAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGTCAATAA	480
	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTTACGGTA	AACTGCCCCA	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCC	CCTGGCATTG	TGCCCAGTAC	ATGACCCTTAT	660
50	GGGACTTTCC	TACTTGCCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	720
	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCAC	TGCTTACTGG	CTTATCGAAA	960
55	TTAATACGAC	TCACTATAGG	GAGACCCAAG	CTTGGTACCA	ATTTAAATTG	ATATCTCCTT	1020
	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGCGGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCCT	TGTTTTAAAA	GGTGTCAGT	1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
	TCTCTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATTA	CATGTATTGG	GTTCCGCCAGA	1260

	CTCCAGAGAA	GAGGCTGGAG	TGGGTCGCAT	ACATTAGTCA	AGGTGGTGAT	ATAACCGACT	1320
	ATCCAGACAC	TGTAAGGGT	CGATTACCA	TCTCCAGAGA	CAATGCCAAG	AACACCCTGT	1380
	ACCTGCAAA	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500
5	CTAGCACCAA	GGGCCCATCG	GTCTTCCCCC	TGGCACCCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCGGTG	ACGGTGTCGT	1620
	GGAACCTAGG	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCTTA	CAGTCCTCAG	1680
	GACTCTACTC	CCTCAGCAGC	GTGGTCACCG	TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCT	1740
	ACATCTGCAA	CGTGAATCAC	AAGCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTTGGTGAGA	1800
10	GGCCAGCACA	GGGAGGGAGG	GTGTCTGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCTGGACG	1860
	CATCCCGGCT	ATGCAGCCCC	AGTCCAGGGC	AGCAAGGCAG	GCCCCGTCTG	CCTCTTCACC	1920
	CGGAGGCCTC	TGCCCCGCCC	ACTCATGCTC	AGGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG	1980
	GCTCTGGGCA	GGCACAGGCT	AGGTGCCCTT	AACCCAGGCC	CTGCACACAA	AGGGGCAGGT	2040
	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC	CGGGAGGACC	CTGCCCTGA	CCTAAGCCCA	2100
15	CCCCAAAGGC	CAAACTCTCC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA	2160
	GTAATCCCA	ATCTTCTCTC	TGCAGAGCCC	AAATCTTGTG	ACAAAACCTCA	CACATGCCCA	2220
	CCGTGCCCCAG	GTAAGCCAGC	CCAGGCCTCG	CCCTCCAGCT	CAAGGCGGGA	CAGGTGCCCT	2280
	AGAGTAGCCT	GCATCCAGGG	ACACACCACG	TGGGTACCAA	CATGTCCGGA	GCCACATGGA	2340
	CAGAGGCCCG	CTCGGCCAC	CCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	CCTCTGTCCC	2400
20	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA	CACCTTGCCC	CCATCCCGGG	ATGAGCTGAC	2460
	CAAGAACCAG	GTCAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCAGCG	ACATCGCCGT	2520
	GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG	ACCACGCCTC	CCGTGCTGGA	2580
	CTCCGACGGC	TCTTCTTCC	TCTACAGCAA	GCTCACCGTG	GACAAGAGCA	GGTGGCAGCA	2640
	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	ACACGCAGAA	2700
25	GAGCCTCTCC	CTGTCTCCGG	GTAAATGAGT	GCGACGGCCG	GCAAGCCCCC	GCTCCCCGGG	2760
	CTCTCGCGGT	CGCACGAGGA	TGCTTGGCAC	GTACCCCTTG	TACATACTTC	CCGGGCGCCC	2820
	AGCATGGAAA	TAAAGCACCC	AGCGCTGCCC	TGGGCCCCTG	CGAGACTGTG	ATGGTTCTTT	2880
	CCACGGGTCA	GGCCGAGTCT	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	GGGTCCCACT	2940
	GTCCCCACAC	TGGCCCAAGC	TGTGCAGGTG	TGCTGGGCC	CCCTAGGGTG	GGGCTCAGCC	3000
30	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	AGCAGCACCT	3060
	GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCTGGG	GACAGACACA	CAGCCCCCTGC	3120
	CTCTGTAGGA	GACTGTCTTG	TTCTGTGAGC	GCCCCGTGTC	TCCCGACCTC	CATGCCCCACT	3180
	CGGGGGCATG	CCTAGTCCAT	GTGCGTAGGG	ACAGGCCCTC	CCTCACCCAT	CTACCCCCAC	3240
	GGCACTAACC	CCTGGCTGCC	CTGCCACGCC	TGCGACCCGC	ATGGGGACAC	AACCGACTCC	3300
35	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GACTGGTGCA	GATGCCCA	CACACACTCA	3360
	GCCCCAGACCC	GTTCAACAAA	CCCCGCACTG	AGGTTGGCCG	GCCACACGGC	CACCACACAC	3420
	ACACGTGCAC	GCCTCACACA	CGGAGCCTCA	CCCCGGCGAA	CTGCACAGCA	CCCAGACCAG	3480
	AGCAAGGTCC	TGCGACACGT	GAACACTCCT	CGGACACAGG	CCCCCACGAG	CCCCACGCGG	3540
	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	TCAGACAAAC	3600
40	CCAGCCCTCC	TCTCACAAGG	GTGCCCTGCG	AGCCGCCACA	CACACACAGG	GGATCACACA	3660
	CCACGTACAG	TCCCTGGCCC	TGGCCCACTT	CCAGTGCCG	CCCTTCCCTG	CAGGACGGAT	3720
	CAGCCTCGAC	TGTGCCTTCT	AGTTGCCAGC	CATCTGTTGT	TTGCCCTCC	CCCGTGCTT	3780
	CCTTGACCCT	GGAAGGTGCC	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	GAAATTGCAT	3840
	CGCATTGTCT	GAGTAGGTGT	CATTCTATTC	TGGGGGTGG	GGTGGGGCAG	GACAGCAAGG	3900
45	GGGAGGATTG	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	ATGGCTTCTG	3960
	AGGCGGAAAG	AACCAGCTGG	GGCTCTAGGG	GGTATCCCCA	CGCGCCCTGT	AGCGGCGCAT	4020
	TAAGCGCGGC	GGGTGTGGTG	GTTACGCGCA	GCGTGACCGC	TACACTTGCC	AGCGCCCTAG	4080
	CGCCCGCTCC	TTTCGCTTTC	TTCCCTTCTC	TTCTCGCCAC	GTTCCGCGGG	CCTCTCAAAA	4140
	AAGGGAAAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	CTAACTCCGC	4200
50	CCATCCCGCC	CCTAACTCCG	CCCAGTTCGG	CCCATTCTCC	GCCCCATGGC	TGACTAATTT	4260
	TTTTTATTTA	TGCAGAGGCC	GAGGCCGCTT	CGGCCTCTGA	GCTATTCCAG	AAGTAGTGAG	4320
	GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	GCTGCGATTT	4380
	CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	CCCCTGCCA	4440
	TCATGGTTCG	ACCATTGAAC	TGCATCGTCG	CCGTGTCCCA	AAATATGGGG	ATTGGCAAGA	4500
55	ACGGAGACCT	ACCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	AGAATGACCA	4560
	CAACCTCTTC	AGTGGAAGGT	AAACAGAATC	TGGTGATTAT	GGGTAGGAAA	ACCTGGTTCT	4620
	CCATTCTCTGA	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	AGTAGAGAAC	4680
	TCAAAGAACC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG	TTTGGATGAT	GCCTTAAGAC	4740
	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	GGAGGCAGTT	4800

	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	ACAAGGATCA	4860
	TGCAGGAATT	TGAAAGTGAC	ACGTTTTTCC	CAGAAATTGA	TTTGGGGAAA	TATAAACTTC	4920
	TCCCAGAATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	AAGTATAAGT	4980
	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	GCTCCCTCC	5040
5	TAAAGCTATG	CATTTTTATA	AGACCATGGG	ACTTTTGCTG	GCTTTAGATC	TCTTTGTGAA	5100
	GGAACCTTAC	TTCTGTGGTG	TGACATAATT	GGACAACTA	CCTACAGAGA	TTTAAAGCTC	5160
	TAAGGTAAAT	ATAAAATTTT	TAAGTGATATA	ATGTGTTAAA	CTACTGATTG	TAATTGTTTG	5220
	TGTATTTTAG	ATTCCAACCT	ATGGAAGTGA	TGAATGGGAG	CAGTGGTGGA	ATGCCCTTAA	5280
	TGAGGAAAAC	CTGTTTTGCT	CAGAAGAAAT	GCCATCTAGT	GATGATGAGG	CTACTGCTGA	5340
10	CTCTCAACAT	TCTACTCCTC	CAAAAAAGAA	GAGAAAGGTA	GAAGACCCCA	AGGACTTTCC	5400
	TTCAGAATTG	CTAAGTTTTT	TGAGTCATGC	TGTGTTTAGT	AATAGAACTC	TTGCTTGCTT	5460
	TGCTATTTAC	ACCACAAAGG	AAAAAGCTGC	ACTGCTATAC	AAGAAAATTA	TGGAAAAATA	5520
	TTCTGTAAAC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	TTTTTCTTAC	5580
	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAAGTATGCT	CAAAAAATTGT	GTACCTTTAG	5640
15	CTTTTAAATT	TGTAAAGGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCCT	TGACTAGAGA	5700
	TCATAATCAG	CCATACCACA	TTGTAGAGG	TTTTACTTGC	TTTAAAAAAC	CTCCACACC	5760
	TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	TTTATTGCAG	5820
	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	GCATTTTTTT	5880
	CACTGCATTG	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	GTCTGGATCG	5940
20	GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCCAC	CCCAACTTGT	6000
	TTATTGCAGC	TTATAATGGT	TACAAATAAA	CAATAAGCAT	CACAAATTTT	ACAAATAAAG	6060
	CATTTTTTTC	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	TCTTATCATG	6120
	TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTTCCTG	6180
	TGTGAAATTG	TTATCCGCTC	ACAATTCAC	ACAACATACG	AGCCGGAAGC	ATAAAGTGTA	6240
25	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	TCACTGCCCG	6300
	CTTCCAGTGC	GGGAAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA	6360
	GAGGCGGTTT	GCGTATTGGG	CGCTCTCCG	CTTCCTCGCT	CACTGACTCG	CTGCGCTCGG	6420
	TCGTTCCGGT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	6480
	AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	6540
30	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	6600
	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	6660
	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	6720
	TGTCCGCCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTCTCA	ATGCTCACGC	TGTAGGTATC	6780
	TCAGTTCCGT	GTAGGTCCGT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	6840
35	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGATC	CAACCCGGTA	AGACACGACT	6900
	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GATTTAGCAG	AGCGAGGTAT	GTAGGCGGTG	6960
	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTTGGTA	7020
	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	7080
	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	7140
40	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAACG	7200
	AAAATCAGC	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	ACCTAGATCC	7260
	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG	7320
	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTTCT	7380
	CCATAGTTGC	CTGACTCCCC	GTCGTGTAGA	TAACACGAT	ACGGGAGGGC	TTACCATCTG	7440
45	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	TTATCAGCAA	7500
	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	TCCGCTCCA	7560
	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCCGCCAGT	AATAGTTTGC	7620
	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACAG	CTCGTCGTTT	GGTATGGCTT	7680
	CATTGAGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTGTGCAAAA	7740
50	AAGCGTTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTGAGAAG	TAAGTTGGCC	GCAAGTGTAT	7800
	CACTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	GTAAGATGCT	7860
	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGATAG	CGGCGACCGA	7920
	GTTGCTCTTG	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	ACTTTAAAAAG	7980
	TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC	GAAAACTCTC	AAGGATCTTA	CCGCTGTTGA	8040
55	GATCCAGTTC	GATGTAACCC	ACTCGTGCAC	CCAAGTATC	TTCAGCATCT	TTTACTTTCA	8100
	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAAG	GGAATAAGGG	8160
	CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCTTTTTTCA	ATATTATTGA	AGCATTTATC	8220
	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG	8280
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(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8897 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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15  GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGCAC CATGAAGTTG CCTGTTAGGC      60
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    CAGTCTCCCT GCCTGTCAGT CTTGGAGATC AAGCGTCCAT CTCTGCAGA TCTAGTCAGA      180
    TCATTGTACA TAATAATGGC AACACCTATT TAGAATGGTA CCTGCAGAAA CCAGGCCAGT      240
    CTCCACAGCT CCTGATCTAC AAAGTTTCCA ACCGATTTTC TGGGGTCCCA GACAGGTTCA      300
    GCGGCAGTGG ATCAGGGACA GATTTCACAC TCAAGATCAG CAGAGTGGAG GCTGAGGATC      360
20  TGGGAGTTTA TTA CTGCTTT CAAGGTTTAC ATGTTCCATT CACGTTCCGC TCGGGGACAA      420
    AGTTGGAAAT AAAACGTAAG TCTCGAGTCT CTAGATAACC GGTCAATCGA TTGGAATTCT      480
    AAACCTGAG GGGGTCCGAT GACGTGGCCA TTCTTTGCCT AAAGCATTGA GTTTACTGCA      540
    AGGTCAGAAA AGCATGCAAA GCCCTGCAAA TGGCTGCAAA GAGCTCCAAC AAAACAATTT      600
    AGAACTTTAT TAAGGAATAG GGGGAAGCTA GGAAGAACT CAAAACATCA AGATTTTAAA      660
25  TACGCTTCTT GGTCTCCTTG CTATAATTAT CTGGGATAAG CATGCTGTTT TCTGTCTGTC      720
    CCTAACATGC CCTTATCCGC AAACAACACA CCAAGGGCA GAACCTTGT ACTTAAACAC      780
    CATCTGTTT GCTTCTTTCC TCAGGAAGTGG TGGCTGCACC ATCTGTCTTC ATCTTCCCGC      840
    CATCTGATGA GCAGTTGAAA TCTGGAAGTGG CCTCTGTGTG GTGCTGTCTG AATAACTTCT      900
    ATCCCAGAGA GGCCAAAGTA CAGTGGAAGG TGGATAACGC CCTCCAATCG GGTAACCTCC      960
30  AGGAGAGTGT CACAGAGCAG GAGAGCAAGG ACAGCACCTA CAGCCTCAGC AGCACCTGTA      1020
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    GCCTGAGCTC GCCCGTCACA AAGAGCTTCA ACAGGGGAGA GTGTTAGAGG GAGAAGTGCC      1140
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35  CCTCCTTGGC TTTAATTATG CTAATGTTGG AGGAGAATGA ATAAATAAAG TGAATCTTTG      1320
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    CAATTTCTCT TATAAGGGAC TAAATATGTA GTCATCCTAA GGCACGTAAC CATTTATAAA      1440
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40  TCCCTCCTC AGCAAGCCCT CATAGTCCTT TTTAAGGGTG ACAGGTCTTA CAGTCATATA      1620
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    TATAAAGAGA ATCATTCAAT GCAACATGAT ATAAATAAAC AACACAATAA AAGCAATTAA      1740
    ATAAACAAAC AATAGGGAAA TGTTTAAGTT CATCATGGTA CTTAGACTTA ATGGAATGTC      1800
    ATGCCTTATT TACATTTTAA AACAGGTACT GAGGGACTCC TGTCTGCCAA GGGCCGTATT      1860
45  GAGTACTTTC CACAACCTAA TTTAATCCAC ACTATACTGT GAGATTAAAA ACATTCATTA      1920
    AAATGTTGCA AAGGTTCTAT AAAGCTGAGA GACAAATATA TTCTATAACT CAGCAATCCC      1980
    ACTTCTAGAT GACTGAGTGT CCCCACCCAC CAAAAAATA TGCAAGAATG TTCAAAGCAG      2040
    CTTTATTTAC AAAAGCCAAA AATTGGAAAT AGCCCCATTG TCCAACAATA GAATGAGTTA      2100
    TTAAACTGTG GTATGTTTAT ACATTAGAAT ACCCAATGAG GAGAATTAAC AAGCTACAAC      2160
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    AAAAGATATG TTCTGTATGT TTTTCATCCAT ATAAAGTTCA AAACCAGGTA AAAATAAAGT      2280
    TAGAAATTTG GATGGAAATT ACTCTTAGCT GGGGGTGGGC GAGTTAGTGC CTGGGAGAAG      2340
    ACAAGAAGGG GCTTCTGGGG TCTTGTTAAT GTTCTGTTCC TCGTGTGGGG TTGTGCAGTT      2400
    ATGATCTGTG CACTGTTCTG TATACACATT ATGCTTCAAA ATAACCTTAC ATAAAGAACA      2460
55  TCTTATACCC AGTTAATAGA TAGAAGAGGA ATAAGTAATA GGTCAAGACC AACGCAGCTG      2520
    GTAAGTGGGG GCCTGGGATC AAATAGCTAC CTGCCTAATC CTGCCWCTT GAGCCCTGAA      2580
    TGAGTCTGCC TTCCAGGGCT CAAGGTGCTC AACAAAACAA CAGGCCTGCT ATTTTCTTGG      2640
    CATCTGTGCC CTGTTTGGCT AGCTAGGAGC ACACATACAT AGAAATTTAA TGAAACAGAC      2700
    CTTACAGCAAG GGGACAGAGG ACAGAATTAA CCTTGCCCAG AACTGGAAA CCCATGTATG      2760

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	ACCTCTCTCT	GCCTACACTC	TGAAGGGGTT	CAGGAGTAAC	TAACACAGCA	TCCCTTCCCT	2940
	CAAATGACTG	ACAATCCCTT	TGTCCTGCTT	TGTTTTTCTT	TCCAGTCAGT	ACTGGGAAAG	3000
5	TGGGGAAGGA	CAGTCATGGA	GAAACTACAT	AAGGAAGCAC	CTTGCCCTTC	TGCCTCTTGA	3060
	GAATGTTGAT	GAGTATCAAA	TCTTTCAAAC	TTTGGAGGTT	TGAGTAGGGG	TGAGACTCAG	3120
	TAATGTCCCT	TCCAATGACA	TGAACCTGGT	CACTCATCCC	TGGGGGCCAA	ATTGAACAAT	3180
	CAAAGGCAGG	CATAATCCAG	TTATGAATTC	TTGCGGCCGC	TTGCTAGCTT	CACGTGTTGG	3240
	ATCCAACCCG	GGAAGGGCCC	TATTCTATAG	TGTCACCTAA	ATGCTAGAGC	TCGCTGATCA	3300
10	GCCTCGACTG	TGCCTTCTAG	TTGCCAGCCA	TCTGTTGTTT	GCCCCCTCCC	CGTGCCTTCC	3360
	TTGACCCTGG	AAGGTGCCAC	TCCCACTGTC	CTTTCCTAAT	AAAATGAGGA	AATTGCATCG	3420
	CATTGTCTGA	GTAGGTGTCA	TTCTATTCTG	GGGGGTGGGG	TGGGGCAGGA	CAGCAAGGGG	3480
	GAGGATTGGG	AAGACAATAG	CAGGCATGGT	GGGGATGCGG	TGGGCTCTAT	GGCTTCTGAG	3540
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	GGAGACCTAC	CCTGGCCTCC	GCTCAGGAAC	GAGTTCAGT	ACTTCCAAAG	AATGACCACA	4140
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25	ATTCTTGAGA	AGAATCGACC	TTTAAAGGAC	AGAATTAATA	TAGTCTCAG	TAGAGAACTC	4260
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30	CCAGAATACC	CAGGCGTCCT	CTCTGAGGTC	CAGGAGGAAA	AAGGCATCAA	GTATAAGTTT	4560
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	AAGCTATGCA	TTTTTATAAG	ACCATGGGAC	TTTTGCTGGC	TTTAGATCTC	TTTGTGAAGG	4680
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35	TATTTTAGAT	TCCAACCTAT	GGAAGTATG	AATGGGAGCA	GTGGTGGAAAT	GCCTTTAATG	4860
	AGGAAAACCT	GTTTTGCTCA	GAAGAAATGC	CATCTAGTGA	TGATGAGGCT	ACTGCTGACT	4920
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	CAGAATTGCT	AAGTTTTTTG	AGTCATGCTG	TGTTTAGTAA	TAGAACTCTT	GCTTGCTTTG	5040
	CTATTACAC	CACAAAGGAA	AAAGCTGCAC	TGCTATACAA	GAAAATTATG	GAAAAATATT	5100
40	CTGTAACCTT	TATAAGTAGG	CATAACAGTT	ATAATCATAA	CATACTGTTT	TTTCTTACTC	5160
	CACACAGGCA	TAGAGTGTCT	GCTATTATAA	ACTATGCTCA	AAAATTGTGT	ACCTTTAGCT	5220
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	ATAATCAGCC	ATACCACATT	TGTAGAGGTT	TTACTTGCTT	TAAAAAACCT	CCACACCTC	5340
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45	TATAATGGTT	ACAAATAAAG	CAATAGCATC	ACAAATTTCA	CAAATAAAGC	ATTTTTTTC	5460
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	TGGATGATCC	TCCAGCGCGG	GGATCTCATG	CTGGAGTTCT	TCGCCCAACC	CAACTTGTTT	5580
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	TGAAATTGTT	ATCCGCTCAC	AATTCCACAC	AACATACGAG	CCGGAAGCAT	AAAGTGTA	5820
	GCCTGGGGTG	CCTAATGAGT	GAGCTAACTC	ACATTAATTG	CGTTGCGCTC	ACTGCCCGCT	5880
	TTCCAGTCGG	GAAACCTGTC	GTGCCAGCTG	CATTAAATGAA	TCGGCCAACG	CGCGGGGAGA	5940
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55	GTTCCGCTGC	GGCGAGCGGT	ATCAGCTCAC	TCAAAGGCGG	TAATACGGTT	ATCCACAGAA	6060
	TCAGGGGATA	ACGCAGGAAA	GAACATGTGA	GCAAAAGGCC	AGCAAAAGGC	CAGGAACCGT	6120
	AAAAAGGCCG	CGTTGCTGGC	GTTTTTCCAT	AGGCTCCGCC	CCCCTGACGA	GCATCAGAAA	6180
	AATCGACGCT	CAAGTCAGAG	GTGGCGAAAC	CCGACAGGAC	TATAAGATA	CCAGGCGTTT	6240
	CCCCCTGGAA	GCTCCCTCGT	GCGCTCTCCT	GTTCCGACCC	TGCCGCTTAC	CGGATACCTG	6300

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	GACCGCTGCG	CCTTATCCGG	TAACTATCGT	CTTAGTCCA	ACCCGGTAAG	ACACGACTTA	6480
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	CAAACCACCG	CTGGTAGCGG	TGGTTTTTTT	GTTTGCAAGC	AGCAGATTAC	GCGCAGAAAA	6720
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	CTCATGGTTA	TGGCAGCACT	GCATAATTCT	CTTACTGTCA	TGCCATCCGT	AAGATGCTTT	7440
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	TGCTCTTGCC	CGGCGTCAAT	ACGGGATAAT	ACCGCGCCAC	ATAGCAGAAC	TTTAAAGATG	7560
	CTCATCATTG	GAAAACGTTT	TTCCGGGCGA	AAACTCTCAA	GGATCTTACC	GCTGTTGAGA	7620
	TCCAGTTTGA	TGTAACCCAC	TCGTGCACCC	AACTGATCTT	CAGCATCTTT	TACTTTTACC	7680
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	TATTTTATTT	TATTTTGTAG	ATGGAGTTTG	GCGCCGATCT	CCCGATCCCC	TATGGTCGAC	8040
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	CGACAATTGC	ATGAAGAATC	TGCTTAGGGT	TAGGCGTTTT	GCGCTGCTTC	GCGATGTACG	8220
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40	CAATGGGCGT	GGATAGCGGT	TTGACTCAGG	GGGATTTCCA	AGTCTCCACC	CCATTGACGT	8700
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45	TAGGGAGACC	CAAGCTT					8897

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8321 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC GGTCAATCGA 60
 TTGGAATTCT TGCGGCCGCT TGCTAGCCAC CATGGAGTTG TGGTTAAGCT TGGTCTTCCT 120

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	TAGTCAAGAT	GGTGATATAA	CCGACTATGC	AGACTCCGTA	AAGGGTCGAT	TCACCATCTC	360
5	CAGAGACAAT	GCAAAGAACA	GCCTGTACCT	GCAAATGAAC	AGCCTGAGGG	ACGAGGACAC	420
	AGCCGTGTAT	TACTGTGCAA	GAGGCCTGGC	GGACGGGGCC	TGGTTTGCTT	ACTGGGGCCA	480
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	ACCCCTCCTCC	AAGAGCACCT	CTGGGGGCAC	AGCGGCCCTG	GGCTGCCTGG	TCAAGGACTA	600
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15	AGAGGGTCTT	CTGGCTTTTT	CCCCAGGCTC	TGGGCAGGCA	CAGGCTAGGT	GCCCCTAACC	1020
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	CTTGTGACAA	AACACACACA	TGCCCAACCT	GCCCCAGGTA	GCCAGCCCAG	GCCTCGCCCT	1260
20	CCAGCTCAAG	GCGGGACAGG	TGCCCTAGAG	TAGCCTGCAT	CCAGGGACAC	ACCACGTGGG	1320
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	GTGACCGCTG	TACCAACCTC	TGTCCCTACA	GGGCAGCCCC	GAGAACCACA	GGTGTACACC	1440
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55	GCTTGGACAG	CTCAGGGCTG	CGATTTTCGG	CCAAACTTGA	CGGCAATCCT	AGCGTGAAGG	3420
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5	AATTGATTTG	GGGAAATATA	AACTTCTCCC	AGAATACCCA	GGCGTCCTCT	CTGAGGTCCA	3960
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	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC	ATAATTGGAC	4140
	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAATATATA	AAITTTTAAG	TGTATAATGT	4200
10	GTAAACTAC	TGATTCTAAT	TGTTTGTGTA	TTTTAGATTG	CAACCTATGG	AACTGATGAA	4260
	TGGGAGCAGT	GGTGGAATGC	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	AGAAATGCCA	4320
	TCAGTGTATG	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	AAAGAAGAGA	4380
	AAGGTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCTAA	GTTTTTTGAG	TCATGCTGTG	4440
	TTTAGTAATA	GAACCTTTCG	TTGCTTTGCT	ATTTACACCA	CAAAGGAAAA	AGCTGCACTG	4500
15	CTATACAAGA	AAATTATGGA	AAAATATTCT	GTAACCTTTA	TAAGTAGGCA	TAACAGTTAT	4560
	AATCATAACA	TACTGTTTTT	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	TATTAATAAC	4620
	TATGCTCAAA	AATTGTGTAC	CTTTAGCTTT	TTAATTTGTA	AAGGGGTTAA	TAAGGAATAT	4680
	TTGATGTATA	GTGCCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTG	TAGAGGTTTT	4740
	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA	TGAATGCAAT	4800
20	TGTTGTTGTT	AACCTGTTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAAGCA	ATAGCATCAC	4860
	AAATTTTACA	AATAAAGCAT	TTTTTTCAC	GCATTCTAGT	TGTGGTTTGT	CCAACTCAT	4920
	CAATGTATCT	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	ATCTCATGCT	4980
	GGAGTTCTTC	GCCCAACCCA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA	AATAAAGCAA	5040
	TAGCATCACA	AATTTTCAAA	ATAAAGCATT	TTTTTCACTG	CATTCTAGTT	GTGGTTTGTG	5100
25	CAAACCTCAT	AATGTATCTT	ATCATGTCTG	TATACCGTCG	ACCTCTAGCT	AGAGCTTGGC	5160
	GTAATCATGG	TCATAGCTGT	TTCTGTGTG	AAATTTGTTT	CCGCTCACAA	TTCCACACAA	5220
	CATACGAGCC	GGAAGCATAA	AGTGTAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAACTCAC	5280
	ATTAATTGCG	TTGCGCTCAC	TGCCCGCTTT	CCAGTCGGGA	AACCTGTCTG	GCCAGCTGCA	5340
	TTAATGAATC	GGCCAAACCG	CGGGGAGAGG	CGGTTTGCCT	ATTGGGCGCT	CTCCGCTTTC	5400
30	CTCGCTCACT	GACTCGCTGC	GCTCGGTCTG	TGGGCTGCGG	CGAGCGGTAT	CAGCTCACTC	5460
	AAAGGCGGTA	ATACGGTTAT	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	5520
	AAAAGGCCAG	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	TTTTCCATAG	5580
	GCTCCGCCCC	CCTGACGAGC	ATCACAATAA	TCGACGCTCA	AGTCAGAGGT	GGCGAAACCC	5640
	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC	TCCCTCGTGC	GCTCTCCTGT	5700
35	TCCGACCCTG	CCGCTTACCG	GATACCTGTC	CGCCTTCTC	CCTTCGGGAA	GCGTGGCGCT	5760
	TTCTCAATGC	TCACGCTGTA	GGTATCTCAG	TTCGGTGTAG	GTCGTTCTGCT	CCAAGCTGGG	5820
	CTGTGTGCAC	GAACCCCCCG	TTACGCCCCG	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT	5880
	TGAGTCCAAC	CCGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG	GTAACAGGAT	5940
	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC	CTAACTACGG	6000
40	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	6060
	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAAAC	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	6120
	TTGCAAGCAG	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	6180
	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	TCATGAGATT	6240
	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATTAATAA	TGAAGTTTAA	AATCAATCTA	6300
45	AAGTATATAT	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	6360
	CTCAGCGATC	TGTCTATTTC	GTTTCATCCAT	AGTTGCCTGA	CTCCCCGTCT	TGTAGATAAC	6420
	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGTCTGA	ATGATACCGC	GAGACCCACG	6480
	CTCACCGGCT	CCAGATTAT	CAGCAATAAA	CCAGCCAGCC	GGAAGGGCCG	AGCGCAGAAG	6540
	TGGTCTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAA	TGTTGCCGGG	AAGCTAGAGT	6600
50	AAGTAGTTCC	CCAGTTAATA	GTTTGCAGAA	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT	6660
	GTCAGGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	6720
	TACATGATCC	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC	TTCGGTCTCT	CGATCGTTGT	6780
	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC	ATAATTCTCT	6840
	TACTGTATG	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	6900
55	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG	CTCTTGCCCC	CGGTCAATAC	GGGATAATAC	6960
	CGCGCCACAT	AGCAGAACTT	TAAAAGTGCT	CATCATTTGA	AAACGTTCTT	CGGGCGGAAA	7020
	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTTCGATG	TAACCCACTC	GTGACCCCAA	7080
	CTGATCTTCA	GCATCTTTTA	CTTTCACCAG	CGTTTCTGGG	TGAGCAAAAA	CAGGAAGGCA	7140
	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	TACTCTTCCT	7200

	TTTTCAATAT	TATTGAAGCA	TTTATCAGGG	TTATTGTCTC	ATGAGCGGAT	ACATATTTGA	7260
	ATGTATTTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	AAGTGCCACC	7320
	TGACGTCGAC	GGATCGGGAG	ATCTGCTAGG	TGACCTGAGG	CGCGCCGCT	TCGAATAGCC	7380
	AGAGTAACCT	TTTTTTTAA	TTTTATTTTA	TTTTATTTT	GAGATGGAGT	TTGGCGCCGA	7440
5	TCTCCCGATC	CCCTATGGTC	GA CTCTCAGT	ACAATCTGCT	CTGATGCCGC	ATAGTTAAGC	7500
	CAGTATCTGC	TCCCTGCTTG	TGTGTTGGAG	GTCGCTGAGT	AGTGCGCGAG	CAAAATTTAA	7560
	GCTACAACAA	GGCAAGGCTT	GACCGACAAT	TGCATGAAGA	ATCTGCTTAG	GGTTAGGCGT	7620
	TTTGCCTGCT	TTCGCGATGT	ACGGGCCAGA	TATACGCGTT	GACATTGATT	ATTGACTAGT	7680
	TATTAATAGT	AATCAATTAC	GGGGTCATTA	GTTTCATAGC	CATATATGGA	GTTCCGCGTT	7740
10	ACATAACTTA	CGGTAAATGG	CCCGCCTGGC	TGACCGCCCA	ACGACCCCG	CCCATTGACG	7800
	TCAATAATGA	CGTATGTTCC	CATAGTAACG	CCAATAGGGA	CTTTCCATTG	ACGTCAATGG	7860
	GTGGACTATT	TACGGTAAAC	TGCCCACTTG	GCAGTACATC	AAGTGTATCA	TATGCCAAGT	7920
	ACGCCCCCTA	TTGACGTCAA	TGACGGTAAA	TGGCCCGCCT	GGCATTATGC	CCAGTACATG	7980
	ACCTTATGGG	ACTTTCCTAC	TTGGCAGTAC	ATCTACGTAT	TAGTCATCGC	TATTACCATG	8040
15	GTGATGCGGT	TTTGGCAGTA	CATCAATGGG	CGTGGATAGC	GGTTTGACTC	ACGGGGATTT	8100
	CCAAGTCTCC	ACCCCATGTA	CGTCAATGGG	AGTTTGTITT	GGCACCAGAA	TCAACGGGAC	8160
	TTTCCAAAAT	GTCGTAACAA	CTCCGCCCCA	TTGACGCAAA	TGGGCGGTAG	GCGTGTACGG	8220
	TGGGAGGTCT	ATATAAGCAG	AGCTCTCTGG	CTAACTAGAG	AACCCACTGC	TTACTGGCTT	8280
20	ATCGAAATTA	ATACGACTCA	CTATAGGGAG	ACCCAAGCTT	G		8321

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8897 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

	GACGGATCGG	GAGATCTGCT	AGCCCCGGTG	ACCTGAGGCG	CGCCGGCTTC	GAATAGCCAG	60
	AGTAACCTTT	TTTTTTAATT	TTATTTTATT	TTATTTTGA	GATGGAGTTT	GGCGCCGATC	120
35	TCCCGATCCC	CTATGGTCGA	CTCTCAGTAC	AATCTGCTCT	GATGCCGCAT	AGTTAAGCCA	180
	GTATCTGCTC	CCTGCTTGTG	TGTTGGAGGT	CGCTGAGTAG	TGCGCGAGCA	AAATTTAAGC	240
	TACAACAAGG	CAAGGCTTGA	CCGACAATTG	CATGAAGAAT	CTGCTTAGGG	TTAGGCGTTT	300
	TGCGCTGCTT	CGCGATGTAC	GGGCCAGATA	TACGCGTTGA	CATTGATTAT	TGACTAGTTA	360
	TTAATAGTAA	TCAATTACGG	GGTCATTAGT	TCATAGCCCA	TATATGGAGT	TCCGCGTTAC	420
40	ATAACTTACG	GTAAATGGCC	CGCCTGGCTG	ACCGCCCAAC	GACCCCGGCC	CATTGACGTC	480
	AATAATGACG	TATGTTCCCA	TAGTAACGCC	AATAGGGACT	TTCCATTGAC	GTCAATGGGT	540
	GGACTATTTA	CGGTAAACTG	CCCACTTGGC	AGTACATCAA	GTGTATCATA	TGCCAAGTAC	600
	GCCCCCTATT	GACGTCAATG	ACGGTAAATG	GCCCCCTGG	CATTATGCCC	AGTACATGAC	660
	CTTATGGGAC	TTTCTACTT	GGCAGTACAT	CTACGTATTA	GTCTACGCTA	TTACCATGGT	720
45	GATGCGGTTT	TGGCAGTACA	TCAATGGGCG	TGGATAGCGG	TTTGACTCAC	GGGGATTTC	780
	AAGTCTCCAC	CCCATTGACG	TCAATGGGAG	TTTGTTTTGG	CACCAAAATC	AACGGGACTT	840
	TCCAAAATGT	CGTAACAAC	CCGCCCAT	GACGCAAATG	GGCGGTAGGC	GTGTACGGTG	900
	GGAGGTCTAT	ATAAGCAGAG	CTCTCTGGCT	AACTAGAGAA	CCCACTGCTT	ACTGGCTTAT	960
	CGAAATTAAT	ACGACTCACT	ATAGGGAGAC	CCAAGCTTGG	TACCAATTTA	AATTGATATC	1020
50	TCCTTAGGTC	TCGAGCACCA	TGAAGTTGCC	TGTTAGGCTG	TTGGTGTCTGA	TGTTCTGGAT	1080
	TCCTGCTTCC	AGCAGTGATG	TTGTCATGAC	CCAAACCCCA	CTGTCCAGTC	CTGTACAGCT	1140
	TGGACAACCT	GCGTCCATCT	CTTGCAATC	TAGTCAGATC	ATTGTACATA	ATAATGGCAA	1200
	CACCTATCTG	GAATGGTACC	AGCAGAGACC	AGGGCAGTCT	CCACGGCTCC	TGATCTACAA	1260
	AGTTTCCAAC	CGATTTTCTG	GGGTCCCGAG	CAGGTTCCAGC	GGCAGTGGAG	CTGGGACAGA	1320
55	TTTCACACTC	AAGATCAGCA	GAGTGGAGGC	TGAGGATGTG	GGAGTTTACT	ACTGCTTCCA	1380
	GGGTTCACAT	GTTCCATTCA	CGTTCCGGCA	AGGGACAAAG	TTGGAATCA	AACGTAAGTC	1440
	TCGAGTCTCT	AGATAACCGG	TCAATCGATT	GGAATTCTAA	ACTCTGAGGG	GGTCCGATGA	1500
	CGTGGCCATT	CTTTGCCTAA	AGCATTGAGT	TTACTGCAAG	GTCAGAAAAG	CATGCAAAGC	1560
	CCTCAGAATG	GCTGCAAAGA	GCTCCAACAA	AACAATTTAG	AACTTTATTA	AGGAATAGGG	1620

	GGAAGCTAGG	AAGAAACTCA	AAACATCAAG	ATTTTAAATA	CGCTTCCTGG	TCTCCTTGCT	1680
	ATAATTATCT	GGGATAAGCA	TGCTGTTTTT	TGCTGTGCCC	TAACATGCCC	TTATCCGCAA	1740
	ACAACACACC	CAAGGGCAGA	ACTTTGTTAC	TTAAACACCA	TCCTGTTTGC	TTCTTTCTCT	1800
5	AGGAACTGTG	GCTGCACCAT	CTGTCTTCAT	CTTCCCGCCA	TCTGATGAGC	AGTTGAAATC	1860
	TGGAAGTGCC	TCTGTTGTGT	GCCTGCTGAA	TAACCTCTAT	CCCAGAGAGG	CCAAAGTACA	1920
	GTGGAAGGTG	GATAACGCCC	TCCAATCGGG	TAACCTCCAG	GAGAGTGTCA	CAGAGCAGGA	1980
	GAGCAAGGAC	AGCACCTACA	GCCTCAGCAG	CACCTGACG	CTGAGCAAAG	CAGACTACGA	2040
	GAAACACAAA	GTCTACGCCT	GCGAAGTCAC	CCATCAGGGC	CTGAGCTCGC	CCGTACACAA	2100
	GAGCTTCAAC	AGGGGAGAGT	GTTAGAGGGA	GAAGTGCCCC	CACCTGCTCC	TCAGTTCCAG	2160
10	CCTGACCCCC	TCCCATCCTT	TGGCCTCTGA	CCCTTTTTTC	ACAGGGGACC	TACCCCTATT	2220
	GCGGTCTCTC	AGCTCATCTT	TCACCTCACC	CCCCTCTCTC	TCCTTGCTCT	TAATTATGCT	2280
	AATGTTGGAG	GAGAATGAAT	AAATAAAGTG	AATCTTTGCA	CCTGTGGTCT	CTCTCTTTCC	2340
	TCATTTAATG	ATTATTATCT	GTTGTTTTAC	CAACTACTCA	ATTTCTCTTA	TAAGGGACTA	2400
	AATATGTAGT	CATCCTAAGG	CACGTAACCA	TTTATAAAAA	TCATCCTTCA	TTCTATTTTA	2460
15	CCCTATCATC	CTCTGCAAGA	CAGTCCTCCC	TCAAACCCAC	AAGCCTTCTG	TCCTCACAGT	2520
	CCCCTGGGCC	ATGGTAGGAG	AGACTTGCTT	CCTTGTTTTT	CCCTCCTCAG	CAAGCCCTCA	2580
	TAGTCCTTTT	TAAGGGTGAC	AGGTCTTACA	GTCATATATC	CTTTGATTCA	ATTCCCTGAG	2640
	AATCAACCAA	AGCAAATTTT	TCAAAGAAG	AAACCTGCTA	TAAAGAGAAT	CATTCATTGC	2700
	AACATGATAT	AAAATAACAA	CACAATAAAA	GCAATTAAAT	AAACAACAA	TAGGGAAATG	2760
20	TTTAAGTTCA	TCATGGTACT	TAGACTTAAT	GGAATGTGCT	GCCTTATTTA	CATTTTAAAA	2820
	CAGGTACTGA	GGGACTCCTG	TCTGCCAAGG	GCCGTATTGA	GTACTTTCCA	CAACCTAATT	2880
	TAATCCACAC	TATACTGTGA	GATTAAAAAC	ATTCAATAAA	ATGTTGCAAA	GGTTCTATAA	2940
	AGCTGAGAGA	CAAATATATT	CTATAACTCA	GCAATCCAC	TTCTAGATGA	CTGAGTGTCC	3000
	CCACCCACCA	AAAAACTATG	CAAGAATGTT	CAAAGCAGCT	TTATTTACAA	AAGCCAAAAA	3060
25	TTGGAAATAG	CCCGATTGTC	CAACAATAGA	ATGAGTTATT	AAACTGTGGT	ATGTTTATAC	3120
	ATTAGAATAC	CCAAATGAGG	GAATTAACAA	GCTACAACTA	TACCTACTCA	CACAGATGAA	3180
	TCTCATAAAA	ATAATGTTAC	ATAAGAGAAA	CTCAATGCAA	AAGATATGTT	CTGTATGTTT	3240
	TCATCCATAT	AAAGTTCAAA	ACCAGGTAAA	AATAAAGTTA	GAAATTTGGA	TGGAAATTAC	3300
	TCTTAGCTGG	GGGTGGGCGA	GTTAGTGCCCT	GGGAGAAGAC	AAGAAGGGGC	TTCTGGGGTC	3360
30	TTGGTAATGT	TCTGTTCCCT	GTGTGGGGTT	GTGCAGTTAT	GATCTGTGCA	CTGTTCTGTA	3420
	TACACATTAT	GCTTCAAAAT	AACTTCACAT	AAAGAACATC	TTATACCCAG	TTAATAGATA	3480
	GAAGAGGAAT	AAGTAATAGG	TCAAGACCAA	CGCAGCTGGT	AAGTGCGGGC	CTGGGATCAA	3540
	ATAGCTACCT	GCCTAATCCT	GCCCWCTTGA	GCCCTGAATG	AGTCTGCCTT	CCAGGGCTCA	3600
	AGGTGCTCAA	CAAAACAACA	GGCCTGCTAT	TTTCTGGCA	TCTGTGCCCT	GTTTGGCTAG	3660
35	CTAGGAGCAC	ACATACATAG	AAATTAAATG	AAACAGACCT	TCAGCAAGGG	GACAGAGGAC	3720
	AGAATTAACC	TTGCCACAGC	ACTGGAAACC	CATGTATGAA	CACTCACATG	TTTGGGAAGG	3780
	GGGAAGGGCA	CATGTAAATG	AGGACTCTTC	CTCATCTCTT	GGGGCACTCT	GGCCCTGCCC	3840
	CTCTCAGCTA	CTCATCCATC	CAACACACCT	TTCTAAGTAC	CTCTCTCTGC	CTACACTCTG	3900
	AAGGGGTTCA	GGAGTAACTA	ACACAGCATC	CCTTCCCTCA	AATGACTGAC	AATCCCTTTG	3960
40	TCCTGCTTTG	TTTTTCTTTC	CAGTCAGTAC	TGGGAAAGTG	GGGAAGGACA	GTCATGGAGA	4020
	AACTACATAA	GGAAGCACCT	TGCCCTTCTG	CCTCTTGAGA	ATGTTGATGA	GTATCAAATC	4080
	TTTCAAACCT	TGGAGGTTTG	AGTAGGGGTG	AGACTCAGTA	ATGTCCCTTC	CAATGACATG	4140
	AACTTGCTCA	CTCATCCCTG	GGGGCCAAAT	TGAACAATCA	AAGGCAGGCA	TAATCCAGTT	4200
	ATGAATCTTT	GCGGCCGCTT	GCTAGCTTCA	CGTGTTGGAT	CCAACCGCGG	AAGGGCCCTA	4260
45	TTCTATAGTG	TCACCTAAAT	GCTAGAGCTC	GCTGATCAGC	CTCGACTGTG	CCTTCTAGTT	4320
	GCCAGCCATC	TGTTGTTTGC	CCCTCCCCCG	TGCCTTCCTT	GACCCTGGAA	GGTGCCACTC	4380
	CCACTGTCCT	TTCTTAATAA	AATGAGGAAA	TGTCATCGCA	TTGTCTGAGT	AGGTGTCATT	4440
	CTATTCTGGG	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA	GACAATAGCA	4500
50	GGCATGCTGG	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAACC	AGCTGGGGCT	4560
	CTAGGGGGTA	TCCCCACGCG	CCCTGTAGCG	GCGCATTAAAG	CGCGGCGGGT	GTGGTGGTTA	4620
	CGCGCAGCGT	GACCGCTACA	CTTGCCAGCG	CCCTAGCGCC	CGCTCCTTTC	GCTTTCTTTC	4680
	CTTCTTTTCT	CGCCACGTTT	GCCGGGCCTC	TCAAAAAAGG	GAAAAAAGC	ATGCATCTCA	4740
	ATTAGTCAGC	AACCATAGTC	CCGCCCCCTA	CTCCGCCCCA	CCCGCCCCCTA	ACTCCGCCCCA	4800
	GTTCCGCCCA	TTCTCCGCCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA	GAGGCCGAGG	4860
55	CCGCCTCGGC	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG	CTTTTTTGGA	GGCCTAGGCT	4920
	TTTGCAAAAA	GCTTGGACAG	CTCAGGGCTG	CGATTTGCGG	CCAAACCTGA	CGGCAATCCT	4980
	AGCGTGAAGG	CTGGTAGGAT	TTTATCCCCG	TGCGCATCAT	GGTTCGACCA	TTGAACTGCA	5040
	TCGTCGCCGT	GTCCCAAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC	5100
	TCAGGAACGA	GTTCAAGTAC	TTCCAAAGAA	TGACCACAAC	CTCTTCAGTG	GAAGGTAAAC	5160

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	TAAAGGACAG	AATTAATATA	GTTCTCAGTA	GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	5280
	ATTTTCTTGC	CAAAGTTTG	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	5340
	GTAAAGTAGA	CATGGTTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAGGAA	GCCATGAATC	5400
5	AACCAGGCCA	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAATTTGAA	AGTGACACGT	5460
	TTTTCCCAGA	AATTGATTG	GGGAAATATA	AACTTCTCCC	AGAATACCCA	GGCGTCTCT	5520
	CTGAGGTCCA	GGAGGAAAAA	GGCATCAAGT	ATAAGTTTGA	AGTCTACGAG	AAGAAAGACT	5580
	AACAGGAAGA	TGCTTTCAAG	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	5640
	CATGGGACTT	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC	5700
10	ATAATTGGAC	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAATATATA	AATTTTAAAG	5760
	TGTATAATGT	GTTAAACTAC	TGATTCTAAT	TGTTTGTGTA	TTTTAGATTCT	CAACCTATGG	5820
	AACTGATGAA	TGGGAGCAGT	GGTGGAATGC	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	5880
	AGAAATGCCA	TCTAGTGATG	ATGAGGCTAT	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	5940
	AAAGAAGAGA	AAGGTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCTAA	GTTTTTTGAG	6000
15	TCATCTGTG	TTTAGTAATA	GAACTCTTGC	TTGCTTTGCT	ATTACACCA	CAAAGGAAAA	6060
	AGCTGCACCT	CTATACAAGA	AAATTATGGA	AAAATATTCT	GTAACCTTTA	TAAGTAGGCA	6120
	TAACAGTTAT	AATCATAACA	TACTGTTTTT	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	6180
	TATTAATAAC	TATGCTCAAA	AATTGTGTAC	CTTTAGCTTT	TTAATTTGTA	AAGGGGTTAA	6240
	TAAGGAATAT	TTGATGTATA	GTGCCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTG	6300
20	TAGAGGTTTT	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA	6360
	TGAATGCAAT	TGTTGTTGTT	AACCTGTTTA	TTGTCAGCTTA	TAATGGTTAC	AAATAAAGCA	6420
	ATAGCATCAC	AAATTTTACA	AATAAAGCAT	TTTTTTCACT	GCATTCTAGT	TGTGGTTTGT	6480
	CCAAACTCAT	CAATGTATCT	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	6540
	ATCTCATGCT	GGAGTTCTTC	GCCCAACCCA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA	6600
25	AATAAAGCAA	TAGCATCACA	AATTTTCAAA	ATAAAGCATT	TTTTTCACTG	CATTCTAGTT	6660
	GTGGTTTGTG	CAAACCTCAT	AATGTATCTT	ATCATGTCTG	TATACCGTCG	ACCTCTAGCT	6720
	AGAGCTTGGC	GTAATCATGG	TCATAGCTGT	TTCTGTGTG	AAATGTTTAT	CCGCTCACAA	6780
	TTCACACAA	CATACGAGCC	GGAAGCATAA	AGTGTAAAGC	CTGGGGTGCC	TAATGAGTGA	6840
	GCTAACTCAC	ATTAATTGCG	TTGCGCTCAC	TGCCCGCTTT	CCAGTCGGGA	AACCTGTCTG	6900
30	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCGT	ATTGGGCGCT	6960
	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	GCTCGGTCTG	TCCGCTGCGG	CGAGCGGTAT	7020
	CAGCTCACTC	AAAGGCGGTA	ATACGGTTAT	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	7080
	ACATGTGAGC	AAAAGGCCAG	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	7140
	TTTTCCATAG	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT	7200
35	GGCGAAACCC	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC	TCCCTCGTGC	7260
	GCTCTCCTGT	TCCGACCCTG	CCGCTTACCG	GATACCTGTC	CGCCTTTCTC	CCTTCGGGAA	7320
	GCGTGCGCT	TTCTCAATGC	TCACGCTGTA	GGTATCTCAG	TTCCGGTGTAG	GTCGTTCCGT	7380
	CCAAGCTGGG	CTGTGTGCAC	GAACCCCCCG	TTTACGCCGA	CCGCTGCGCC	TTATCCGGTA	7440
	ACTATCGTCT	TGAGTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG	7500
40	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC	7560
	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG	AAGCCAGTTA	7620
	CCCTCGGAAA	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAACA	AACCACCGCT	GGTAGCGGTG	7680
	GTTTTTTTGT	TTGCAAGCAG	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	7740
	TGATCTTTTC	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	7800
45	TCATGAGATT	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATTAAAAA	TGAAGTTTTA	7860
	AATCAATCTA	AAGTATATAT	GAGTAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	7920
	AGGCACCTAT	CTCAGCGATC	TGTCTATTTT	GTTTATCCAT	AGTTGCCTGA	CTCCCCGTCG	7980
	TGTAGATAAC	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA	ATGATACCGC	8040
	GAGACCCACG	CTCACCCTG	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGAAGGGCCG	8100
50	AGCGCAGAAG	TGGTCTTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	8160
	AAGCTAGAGT	AAGTAGTTCG	CCAGTTAATA	GTTTGCAGAA	CGTTGTTGCC	ATTGCTACAG	8220
	GCATCGTGGT	GTCACGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	8280
	CAAGGCGAGT	TACATGATCC	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC	TTCCGTCCTC	8340
	CGATCGTTGT	CAGAAGTAAG	TTGGCCGCGT	TGTTTACTCT	CATGGTTATG	GCAGCACTGC	8400
55	ATAATTCTCT	TACTGTCTATG	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	8460
	CCAAGTCATT	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG	CTCTTGCCCG	GCGTCAATAC	8520
	GGGATAATAC	CGCGCCACAT	AGCAGAACTT	TAAAAGTGCT	CATCATTGGA	AAACGTTCTT	8580
	CGGGGCGAAA	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTCGATG	TAACCCACTC	8640
	GTGCACCCAA	CTGATCTTCA	GCATCTTTTA	CTTTCACCAT	CGTTTCTGGG	TGAGCAAAAA	8700

CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	8760
TACTCTTCCT	TTTTCAATAT	TATTGAAGCA	TTTATCAGGG	TTATTGTCTC	ATGAGCGGAT	8820
ACATATTTGA	ATGTATTTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	8880
AAGTGCCACC	TGACGTC					8897

What is claimed is:

1. A method for inhibiting immunoglobulin-induced toxicity resulting from
5 immunoglobulin immunotherapy in a subject comprising administering an
immunoglobulin molecule to the subject, the immunoglobulin molecule
having a variable region and a constant region, the immunoglobulin molecule
being modified prior to administration by structurally altering multiple
toxicity associated domains in the constant region so that immunoglobulin-
10 induced toxicity is inhibited.
2. A method for inhibiting immunoglobulin-induced toxicity resulting from
immunoglobulin immunotherapy in a subject comprising administering a
structurally altered antibody to the subject, the structurally altered antibody
15 comprising a variable region and a constant region, multiple toxicity
associated domains in the constant region being modified so as to render the
constant region unable to mediate an ADCC response or activate
complement thereby inhibiting immunoglobulin-induced toxicity resulting
from immunotherapy.
- 20 3. A method for inhibiting immunoglobulin-induced toxicity resulting from
immunotherapy in a subject comprising administering an Ig fusion protein to
the subject, the Ig fusion protein having multiple structurally altered toxicity
associated domains in the constant region.
- 25 4. A method for inhibiting immunoglobulin-induced toxicity resulting from
immunotherapy in a subject comprising administering an Ig fusion protein to
the subject, the Ig fusion protein comprising a modified constant region, the

modification being a structural alteration in multiple toxicity associated regions within the CH₂ domain.

5. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
- (a) selecting an immunoglobulin which recognizes and binds a target, the target being associated with the disease;
 - (b) mutating the immunoglobulin so selected by structurally altering multiple toxicity associated domains in the constant region of the immunoglobulin thereby creating a structurally altered immunoglobulin;
 - (c) administering the structurally altered immunoglobulin of step (b) to the subject under conditions so that the structurally altered immunoglobulin recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the constant region thereby preventing immunoglobulin-induced toxicity in the subject.
6. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
- (a) selecting an Ig fusion protein which recognizes and binds a target, the target being associated with the disease;
 - (b) structurally altering multiple toxicity associated domains in the CH₂ domain of the constant region of the Ig protein so selected;

- 5 (c) administering the structurally altered Ig fusion protein of step (b) to the subject under conditions so that the structurally altered Ig fusion protein recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the CH₂ domain thereby preventing immunoglobulin-induced toxicity in the subject.
- 10 7. The method of claim 1, 2, 3, 4, 5, or 6, wherein the portion of the constant region is the CH₂ domain.
8. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgG.
9. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgM.
- 15 10. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgA.
11. The method of claim 2, wherein the antibody recognizes and binds Le^y.
- 20 12. The method of claim 2, wherein the antibody recognizes and binds to Le^x.
13. The method of claim 2, wherein the antibody is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
- 25 14. The method of claim 2, wherein the antibody is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.

15. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds Le^y.
- 5 16. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds to Le^x.
17. The method of claim 1 or 5, wherein the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
- 10 18. The method of claim 1 or 5, wherein the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
- 15 19. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds Le^y.
20. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds to Le^x.
- 20 21. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
- 25 22. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
23. A pharmaceutical composition comprising a pharmaceutically effective

amount of a structurally altered immunoglobulin, and an acceptable carrier, the structurally altered immunoglobulin (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.

5 24. A pharmaceutical composition comprising a pharmaceutically effective amount of structurally altered Ig fusion protein, and an acceptable carrier, the structurally altered Ig fusion protein (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.

10 25. A method of treating carcinomas in vivo comprising administering to a subject a pharmaceutically effective amount of the composition of claim 23 or 24.

15 26. The method of claim 30, wherein the structurally altered immunoglobulin in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.

20 27. The method of claim 24, wherein the Ig fusion protein in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.

25 28. The method of claim 2 or 5, wherein the antibody is conjugated to a cytotoxic agent.

29. The method of claim 1, wherein the immunoglobulin is conjugated to a cytotoxic agent.

30. The method of claim 3, 4 or 6, wherein the Ig fusion protein is conjugated to a cytotoxic agent.
31. The method of claim 28, 29, or 31, wherein the cytotoxic agent is selected
5 from the group consisting of antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents.
32. A method for treating a subject suffering from a cancer, the cancer being
10 characterized as a group of cells having a tumor associated antigen on the cell surface, which method comprises administering to the subject a cancer killing amount of the composition of claim 23 or 24 joined to a cytotoxic agent under conditions which permit the molecule so joined to bind the tumor associated antigen on the cell surface so as to kill the cells so bound
15 thereby curing the subject.
33. A pharmaceutical composition comprising a pharmaceutically effective amount of a structurally altered BR96 antibody, the structurally altered antibody having an inactivated CH₂ domain.
20
34. A method for treating a subject suffering from a proliferative type disease characterized by cells having a BR96 antigen on the cell surface which comprises administering to the subject an effective amount of the composition of claim 33 joined to doxorubicin such that the
25 immunoconjugate binds the BR96 antigen and kills said cells thereby treating the subject.
35. A method for inhibiting BR96 (ATCC: HB10036) induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering

BR96 to the subject, the BR96 molecule being modified prior to administration, the modification comprising the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated toxicity is inhibited.

36. A method for preventing BR96 (ATCC: HB10036) induced toxicity resulting from immunotherapy for cancer in a subject comprising:
- (a) mutating the BR96 polypeptide by the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated immunoglobulin-induced toxicity is inhibited in the altered BR96 polypeptide; and
 - (b) administering the structurally altered BR96 polypeptide of step (a) to the subject under conditions so that the peptide recognizes and binds cancer associated Le^y antigens, thereby alleviating symptoms associated with the cancer, the structural alteration of the toxicity associated domains thereby preventing BR96 toxicity in the subject.
37. A chimeric BR96 antibody having a structurally altered constant region having the CH1 and CH3 domains but not the CH2 domain, the antibody being designated cBR96-A.

38. The chimeric BR96 antibody of claim 37 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 10.
39. A BR96 antibody having humanized variable and constant regions, wherein the constant region has been structurally altered so that the CH1 and CH3 domains are present but the CH2 domain is not, the antibody being designated hBR96-2A.
40. The BR96 antibody of claim 39 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 12.
41. A BR96 antibody designated hBR96-2B having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine and glycine at amino acid position 237 is mutated to alanine.
42. A BR96 antibody designated hBR96-2C having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.
43. A BR96 antibody designated hBR96-2D having a structurally altered constant region wherein proline at amino acid position 331 is mutated to alanine.
44. A BR96 antibody designated hBR96-2E having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid

position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.

- 5 45. A BR96 antibody designated hBR96-2F having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; and proline at amino acid position 331 is mutated to alanine.
- 10 46. A BR96 antibody designated hBR96-2G having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
- 15 47. A BR96 antibody designated hBR96-2H having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
- 20
48. A nucleic acid molecule which encodes the BR96 antibody of claim 37, 39, and 41-47.
- 25
49. A cDNA of claim 48.
50. A plasmid which comprises the nucleic acid molecule of claim 48.

51. A host vector system comprising a plasmid of claim 50 in a suitable host cell.
52. A method for producing a protein comprising growing the host vector system of claim 51 so as to produce the protein in the host and recovering the protein so produced.
- 5

molecule's possibility of mediating antibody-dependent cellular cytotoxicity (ADCC), (2) bind C1q, or (3) activate complement.

Alternatively, structural alteration can be effected by deletion of only that portion of
5 the CH₂ domain that binds the Fc receptor or complement.

Further alternatively, a single mutation or multiple mutations such as substitutions and insertions in the CH₂ domain can be made. The underlying requirement of any mutation is that it must inhibit, diminish, or block immunoglobulin-induced toxicity.

10 For example, this can be achieved by mutating the constant region such that the altered molecule is rendered unable to mediate a CDC response or an ADCC response, or to activate complement.

Alternatively, structural alteration can be effected by isotype switching (also known
15 as class switching) so that the altered molecule does not induce toxicity in the subject. In one embodiment, the constant region of the immunoglobulin is structurally altered so that it no longer binds the Fc receptor or a complement component, e.g., switching a molecule's original IgG isotype from IgG1 to IgG4. Isotype switching can be effected regardless of species, i.e., an isotype from a non-
20 human being can be switched with an isotype from a human being (E.D. Finkelman et al. (1990) Annu. Rev. Immunol. 8:303-333; T. Honjo et al. (1979) Cell 18: 559-568; T. Honjo et al. In "Immunoglobulin Genes" pp. 124-149 Academic Press, London)).

25 As used herein the term "Ig fusion protein" means any recombinantly produced antigen or ligand binding domain having a constant region which can be structurally altered.

As used herein "cytotoxic agent" includes antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents. Specific examples within these groups include but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, supporin, gelonin, PE40, bryodin, dihydroxy anthracin dione, actinomycin D, and 1-dehydrotestosterone.

As used herein the term "BR96" refers to (1) the whole BR96 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, (2) chimeric BR96 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, or (3) BR96 mutant molecules disclosed in PCT No. 95/305444, published March 6, 1996.

As used herein, "treating" means to (1) provide tumor regression so that the tumor is not palpable for a period of time (standard tumor measurement procedures may be followed (A.B. Miller et al. "Reporting results of cancer treatment" Cancer 47:207-214 (1981)); (2) stabilize the disease; or (3) provide any clinically beneficial effects.

As used herein, an "effective amount" is an amount of the antibody, immunoconjugate, or recombinant molecule which kills cells or inhibits the proliferation thereof.

As used herein, "administering" means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular or subcutaneous administration, or the implantation of a slow-release device such as a miniosmotic pump, to the subject.

As used herein, "pharmaceutically acceptable carrier" includes any material which when combined with the antibody retains the antibody's specificity or efficacy and is

non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including
5 coated tablets and capsules.

Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may
10 also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods.

As used herein, "mutation" means a single amino acid or nucleic acid mutation or multiple mutations by whatever means, e.g., homologous recombination, error prone
15 PCR, or site directed mutagenesis.

In order that the invention herein described may be more fully understood, the following description is set forth.

20 METHODS OF THE PRESENT INVENTION

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from the use of immunoglobulin during therapy or in vivo diagnosis. For example, the methods of the invention would be useful to minimize
25 the toxicity associated with prolonged clinical exposure to immunoglobulin use during or after tumor imaging with radiolabeled antibodies.

In accordance with the practice of this invention, the subject includes, but is not limited to, human, equine, porcine, bovine, murine, canine, feline, and avian

subjects. Other warm blooded animals are also included in this invention.

This method comprises administering an immunoglobulin molecule to the subject. The immunoglobulin can be IgG, IgM, or IgA. IgG is preferred.

5

In one embodiment of the invention, the immunoglobulin molecule recognizes and binds Le^y. In another embodiment, the immunoglobulin recognizes and binds Le^x.

In a further embodiment, the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma deposited on February 22, 1989 with the American Type
10 Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and
accorded ATCC Accession No.: HB 10036. In yet another embodiment, the
immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma
deposited on May 23, 1990, with the ATCC, 12301 Parklawn Drive, Rockville, MD
20852 and accorded ATCC Accession No.: HB 10460.

15

In accordance with the practice of the invention, the immunoglobulin can be a
bispecific antibody with a binding specificity for two different antigens, one of the
antigens being that with which the monoclonal antibody BR96 produced by the
hybridoma having the identifying characteristics of HB 10036 as deposited with the
20 ATCC binds. Also, in accordance with the practice of the invention, the
immunoglobulin can be an anti-idiotypic antibody.

As required by the invention, at least a portion of the constant region of the
immunoglobulin molecule is structurally altered. Structural alteration can be
25 effected by a number of means. In one embodiment, the entire constant region, i.e.,
CH₁, CH₂, and CH₃ domains, can be deleted.

In another embodiment, only the CH₂ domain is deleted from the immunoglobulin
molecule (e.g., cBR96-A (Figure 5), hBR96-2A (Figure 4). In this embodiment, the

CH₂ deletion may result in a molecule unable to bind the Fc γ receptor or a complement component.

In another embodiment, only that portion of the CH₂ domain which binds the complement component C1q is deleted. In yet another embodiment, mutations in specific portions of the CH₂ domain are made. For example, the immunoglobulin molecule may be modified by structurally altering multiple toxicity associated domains in the constant region so that immunoglobulin-induced toxicity is inhibited. A discussion of such mutations are further found hereinafter.

10

Regardless of the means, the underlying requirement for any structural alteration of the constant region is that immunoglobulin-induced toxicity is substantially reduced or inhibited. In one embodiment, immunoglobulin-induced toxicity is inhibited by structurally altering the constant region such that the molecule's ability to mediate a CDC response or ADCC response and/or activate the complement cascade is prevented or inhibited. Methods for determining whether the molecule is able to inhibit a CDC response are well known, e.g., one method involves a ⁵¹Cr-release test (H. Garrigues et al. Int. J. Cancer 29:511 (1982); I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to inhibit an ADCC response are well known (I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to activate a complement cascade are well known.

15
20

In another embodiment of the invention, the method comprises administering to the subject an Ig fusion protein having a structurally altered constant region. Structural alteration of the constant region may include deletion of the entire C region or portions thereof, e.g., alteration of the CH₂ domain so that the altered molecule no longer binds the Fc receptor or a complement component.

25

The invention further provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject. The method comprises administering to the subject an antibody which has been modified so that at least a portion of the constant region has been structurally altered as discussed supra. In one
5 embodiment, the antibody recognizes and binds Le^y. In another embodiment, the antibody recognizes and binds to Le^x.

In accordance with the practice of this invention, the antibody can be monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of
10 HB 10036 as deposited with the ATCC. Alternatively, the antibody can be chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC. Further, the antibody can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma
15 having the identifying characteristics of HB 10036 as deposited with the ATCC binds.

Additionally, the present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a
20 subject. The disease will vary with the antigen sought to be bound. Examples of diseases include but are not limited to immunological diseases, cancer, cardiovascular diseases, neurological diseases, dermatological diseases or kidney disease.

25 This method comprises the following steps. Step one provides selecting an antibody for a target. Generally, the target is associated with the disease and the antibody directed to the target is known. For example, the target can be the BR96 antigen and the antibody selected is BR96.

Step two of this method provides structurally altering the constant region of the antibody so selected so that immunoglobulin induced toxicity is inhibited. Inactivation can include any of the means discussed above. For example, inactivation can be effected by structurally altering multiple toxicity associated domains in the CH₂ domain of the constant region of the Ig protein so selected.

Step three of this method provides administering the structurally altered antibody of step two to the subject under conditions that the structurally altered antibody recognizes and binds the target and that such binding directly or indirectly alleviates symptoms associated with the disease.

In accordance with the invention, in one embodiment step one provides selecting an Ig fusion protein for a target. Further, the method provides mutating the Ig fusion protein so selected by structurally altering the CH₂ domain of the constant region of the Ig protein by the same means discussed above.

The invention further provides methods to treat human carcinoma. For example, the immunoglobulin, antibody, or Ig fusion protein discussed above can be used in combination with standard or conventional treatment methods such as chemotherapy, radiation therapy or can be conjugated or linked to a therapeutic drug, or toxin, as well as to a lymphokine or a tumor-inhibitory growth factor, for delivery of the therapeutic agent to the site of the carcinoma.

Techniques for conjugating therapeutic agents to immunoglobulins are well known (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellström et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In

Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)).

5

Alternatively, the structurally altered antibody or Ig fusion protein can be coupled to high-energy radiative agents, e.g., a radioisotope such as ^{131}I ; which, when localized at the tumor site, results in a killing of several cell diameters (see, e.g., Order, "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985)). According to yet another embodiment, the structurally altered BR96 antibody can be conjugated to a second antibody to form an antibody heteroconjugate for the treatment of tumor cells as described by Segal in United States Patent 4,676,980.

15

Still other therapeutic applications for the structurally altered antibody or Ig fusion protein of the invention include conjugation or linkage, e.g., by recombinant DNA techniques or protein chemical techniques, to an enzyme capable of converting a prodrug into a cytotoxic drug and the use of that antibody-enzyme conjugate in combination with the prodrug to convert the prodrug to a cytotoxic agent at the tumor site (see, e.g., Senter et al., "Anti-Tumor Effects Of Antibody-alkaline Phosphatase", Proc. Natl. Acad. Sci. USA, 85:4842-46 (1988); "Enhancement of the in vitro and in vivo Antitumor Activities of Phosphorylated Mitomycin C and Etoposide Derivatives by Monoclonal Antibody-Alkaline Phosphatase Conjugates", Cancer Research 49:5789-5792 (1989); and Senter, "Activation of Prodrugs by Antibody-Enzyme Conjugates: A New Approach to Cancer Therapy," FASEB J. 4:188-193 (1990)).

It is apparent therefore that the present invention encompasses pharmaceutical compositions including immunoglobulin molecules, antibodies, and Ig fusion proteins all having structurally altered CH₂ domains, and their use in methods for treating human carcinomas. For example, the invention includes pharmaceutical compositions for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of a structurally altered BR96 and a pharmaceutically acceptable carrier.

The compositions may contain the structurally altered antibody or Ig fusion protein or antibody fragments, either unmodified, conjugated to a therapeutic agent (e.g., drug, toxin, enzyme or second antibody). The compositions may additionally include other antibodies or conjugates for treating carcinomas (e.g., an antibody cocktail).

The compositions of the invention can be administered using conventional modes of administration including, but not limited to, intrathecal, intravenous, intraperitoneal, oral, intralymphatic or administration directly into the tumor. Intravenous administration is preferred.

The composition of the invention can be in a variety of dosage forms which include, but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

The compositions of the invention also preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate.

In accordance with the practice of the invention, the pharmaceutical carrier can be a lipid carrier. The lipid carrier can be a phospholipid. Further, the lipid carrier can be a fatty acid. Also, the lipid carrier can be a detergent. As used herein, a detergent
5 is any substance that alters the surface tension of a liquid, generally lowering it.

In one example of the invention, the detergent can be a nonionic detergent. Examples of nonionic detergents include, but are not limited to, polysorbate 80 (also known as Tween 80 or (polyoxyethylenesorbitan monooleate), Brij, and Triton (for
10 example Triton WR-1339 and Triton A-20).

Alternatively, the detergent can be an ionic detergent. An example of an ionic detergent includes, but is not limited to, alkyltrimethylammonium bromide.

15 Additionally, in accordance with the invention, the lipid carrier can be a liposome. As used in this application, a "liposome" is any membrane bound vesicle which contains any molecules of the invention or combinations thereof.

The most effective mode of administration and dosage regimen for the compositions
20 of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician.

The interrelationship of dosages for animals of various sizes and species and humans based on mg/m^2 of surface area is described by Freireich, E.J., et al. Cancer
25 Chemother., Rep. 50 (4): 219-244 (1966). Adjustments in the dosage regimen can be made to optimize the tumor cell growth inhibiting and killing response, e.g., doses can be divided and administered on a daily basis or the dose reduced proportionally depending upon the situation (e.g., several divided doses can be

administered daily or proportionally reduced depending on the specific therapeutic situation).

THE MOLECULES OF THE INVENTION

5

The present invention provides structurally altered BR96 or BR96 Ig fusion proteins.

Structurally altered BR96 antibodies or Ig fusion proteins have the variable region of BR96 and a modified constant region. This modification provides structurally altered BR96 antibodies or Ig fusion proteins with the ability to inhibit
10 immunoglobulin-induced toxicity.

Various embodiments of structurally altered BR96 or BR96 Ig fusion proteins have been made.

15 In one embodiment, designated cBR96-A, the entire CH₂ domain of cBR96 was deleted. CBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 10. cBR96 is expressed by a plasmid having the sequence in SEQ ID NO. 9.

20 In another embodiment, designated hBR96-2A, the entire CH₂ domain of hBR96 was deleted. hBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 12. hBR96 is a mutant BR96 having the H1, H2, and H3 mutations described in PCT Application No. 95/305444, published March 6, 1996.

25 In yet another embodiment, designated hBR96-2B, the leucine residue located at amino acid position 235 is mutated to alanine. Additionally, the glycine residue located at amino acid position 237 is mutated to alanine. The amino acid position numbering used is described in Kabat et al. Sequences of Proteins of Immunological Interest 5th Edition (1991) United States Department of Health and Human Services.

In a further embodiment, designated hBR96-2C, the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine
5 using standard protocols (Alexander R. Duncan and Greg Winter "The binding site for C1q on IgG" Nature 332:738 (1988)).

In another embodiment, designated hBR96-2D, the proline residue at position 331 is mutated to alanine (M-H. Tao et al., "Structural features of human immunoglobulin
10 G that determine isotype-specific differences in complement activation" J. Exp. Med. 178:661-667 (1993); Y. Xu et al., "Residue at position 331 in the IgG1 and IgG4 domains contributes to their differential ability to bind and activate complement" J. Biol. Chem. 269:3469-3474 (1994)).

15 In an additional embodiment, designated hBR96-2E, the leucine residue at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine (A. Morgan et al., "The N-terminal end
20 of the CH₂ domain of chimeric human IgG1 anti-HLA-DR is necessary for C1q, Fc(gamma)RI and Fc(gamma)RIII binding" Immunol. 86:319-324 (1995)).

In yet a further embodiment, designated hBR96-2F, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is
25 mutated to alanine; and the proline residue located at position 331 is mutated to alanine.

In yet another embodiment, designated hBR96-2G, the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is

mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

In another embodiment, designated hBR96-2H, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

10

Depending on its form, a structurally altered BR96 antibody or fusion protein can be a monofunctional antibody, such as a monoclonal antibody, or bifunctional antibody, such as a bispecific antibody or a heteroantibody. The uses of structurally altered BR96, i.e., as a therapeutic or diagnostic agent, will determine the different forms of structurally altered BR96 which is made.

15

Several options exist for antibody expression. Immunoexpression libraries can be combined with transfectoma technology, i.e., the genes for the Fab molecules derived from the immunoglobulin gene expression library can be connected to the desired constant-domain exons. These recombinant genes can then be transfected and expressed in a transfectoma that would secrete an antibody molecule.

20

Once produced, the polypeptides of the invention can be modified, i.e., by amino acid modifications within the molecule, so as to produce derivative molecules. Such derivative molecules would retain the functional property of the polypeptide, namely, the molecule having such substitutions will still permit the binding of the polypeptide to the BR96 antigen or portions thereof.

25

It is a well-established principle of protein chemistry that certain amino acid

substitutions, entitled "conservative amino acid substitutions," can frequently be made in a protein without altering either the conformation or the function of the protein.

- 5 Amino acid substitutions include, but are not necessarily limited to, amino acid substitutions known in the art as "conservative".

Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid
10 (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa.

Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional
15 structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine and valine (V).

Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R)
20 are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

- 25 In one embodiment of the present invention, the polypeptide is substantially pure, i.e., free of other amino acid residues which would inhibit or diminish binding of the polypeptide to its target and would inhibit or reduce gastrointestinal toxicity which are normally exhibited during or after antibody therapy.

NUCLEIC ACID MOLECULES ENCODING THE PRESENT INVENTION

The nucleotide sequences and the amino acid sequences of the variable and constant regions of BR96 are known. The sequence for the immunoglobulin constant region is known and provided in Figure 18. Specific mutations in the constant region of the BR96 antibody were made. Nucleic acid molecules encoding the seven mutants described above (hBR96-2B through hBR96-2H) are as follows.

In hBR96-2B, alanine at amino acid positions 235 and 237 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2C, serine at positions 318, 320, and 322 is encoded by UCU, UCC, UCA, or UGG.

In hBR96-2D, alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2E, alanine at positions 235 and 237 is encoded by codons GCU, GCC, GCA, or GCG. Serine at positions 318, 320, and 322 is encoded by UCU, UCC, UCA, or UGG.

In hBR96-2F, alanine at positions 235, 237, and 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2G, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG. Further, the alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2H, alanine at positions 235, 237, and 331 is encoded by codons GCU,

GCC, GCA, or GCG. Additionally, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG.

Any of the above can be deoxyribonucleic acid (DNA), e.g., complementary DNA
5 (cDNA), or ribonucleic acid (RNA).

IMMUNOCONJUGATES

Immunoconjugates (having whole antibody or Ig fusion proteins) may be
10 constructed using a wide variety of chemotherapeutic agents such as folic acid and anthracyclines (Peterson et al., "Transport And Storage Of Anthracyclines In Experimental Systems And Human Leukemia", in Anthracycline Antibiotics In Cancer Therapy, Muggia et al. (Eds.), p. 132 (Martinus Nijhoff Publishers (1982); Smyth et al., "Specific Targeting of Chlorambucil to Tumors With the Use of
15 Monoclonal Antibodies", J. Natl. Cancer Inst., 76:503-510 (1986)), including doxorubicin (DOX) (Yang and Reisfeld "Doxorubicin Conjugated with a Monoclonal Antibody Directed to a Human Melanoma-Associated Proteoglycan Suppresses Growth of Established Tumor xenografts in Nude Mice PNAS (USA)" 85:1189-1193 (1988)), Daunomycin (Arnon and Sela "In Vitro and in vivo Efficacy
20 of Conjugates of Daunomycin With Anti-Tumor Antibodies" Immunol. Rev., 65:5-27 (1982)), and morpholinodoxorubicin (Mueller et al., "Antibody Conjugates With Morpholinodoxorubicin and Acid-Cleavable Linkers", Bioconjugate Chem., 1:325-330 (1990)).

25 BR96 has been conjugated to doxorubicin and has been shown to be effective in therapy of certain cancers or carcinomas (Trail, P.A., Willner, D., Lasch, S.J., Henderson, A.J., Casazza, A.M., Firestone, R.A., Hellström, I., and Hellström, K.E. Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. Science, 261:212-215, 1993).

In accordance with the practice of the invention, structurally altered BR96 can be used in forms including unreduced IgG, reduced structurally altered IgG, and fusion proteins (PCT Application No. 95/305444, published March 6, 1996).

5

Suitable therapeutic agents for use in making the immunoconjugate includes Pseudomonas exotoxin A (PE) in either the native PE or LysPE40 form. LysPE40 is a truncated form containing a genetically modified amino terminus that includes a lysine residue for conjugation purposes. Doxorubicin is also a suitable therapeutic

10 agent.

Additional examples of therapeutic agents include, but are not limited to, antimetabolites, alkylating agents, anthracyclines, antibiotics, and anti-mitotic agents.

15

Antimetabolites include methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine.

20

Alkylating agents include mechlorethamine, thiotepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin.

25

Anthracyclines include daunorubicin (formerly daunomycin) and doxorubicin (also referred to herein as adriamycin). Additional examples include mitozantrone and bisantrene.

Antibiotics include dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC).

Antimitotic agents include vincristine and vinblastine (which are commonly referred to as vinca alkaloids).

- 5 Other cytotoxic agents include procarbazine, hydroxyurea, asparaginase, corticosteroids, mytostane (O,P'-(DDD)), interferons.

- Further examples of cytotoxic agents include, but are not limited to, ricin, bryodin, gelonin, supporin, doxorubicin, taxol, cytochalasin B, gramicidin D, ethidium
10 bromide, etoposide, teniposide, colchicine, dihydroxy anthracin dione, 1-dehydrotestosterone, and glucocorticoid.

- Clearly analogs and homologs of such therapeutic and cytotoxic agents are encompassed by the present invention. For example, the chemotherapeutic agent
15 aminopterin has a correlative improved analog namely methotrexate.

- Further, the improved analog of doxorubicin is an Fe-chelate. Also, the improved analog for 1-methylnitrosourea is lomustine. Further, the improved analog of vinblastine is vincristine. Also, the improved analog of mechlorethamine is
20 cyclophosphamide.

METHODS FOR MAKING MOLECULES OF THE INVENTION

- There are multiple approaches to making site specific mutations in the CH₂ domain
25 of an immunoglobulin molecule. One approach entails PCR amplification of the CH₂ domain with the mutations followed by homologous recombination of the mutated CH₂ into the vector containing the desired immunoglobulin, e.g., hBR96-2. For example, hBR96-2B and hBR96-2D have been made by this method.

Another approach would be to introduce mutations by site-directed mutagenesis of single-stranded DNA. For example, vector pD17-hG1b, which contains only the constant region of IgG1 and not the V domain of hBR96, has the fl origin of replication. This gives the vector the properties of a phagemid and site-directed mutagenesis experiments can be performed according to the methods of Kunkel, et al. (Kunkel, T.A., J.D. Roberts, and R.A. Zakour, 1987 Methods Enzymol. 154:367-383) as provided in the Bio-Rad Muta-Gene® phagemid *in vitro* mutagenesis kit, version 2. For example, hBR96-2B, -C, -D, -E, -F, -G, and -H were made by this method.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the scope of this invention in any manner.

EXAMPLE 1

The following standard ELISA protocol was used.

Materials: Immulon2 96 well plates and Genetic Systems Specimen Diluent Concentrate (10x); antibody conjugate was Goat Anti Human Kappa-HRP Mouse Adsorbed, Southern Biotech. at 1:10,000 in Genetic Systems Conjugate Diluent (1x); Genetic Systems EIA Chromogen Reagent (TMB) (1:100); Genetic Systems EIA Buffered Substrate (1x); primary antibody or antigen were AffiniPure F(ab')₂ Fragment Goat Anti Human IgG Fc Fragment specific (Jackson Immuno Research), Goat Anti Human Kappa-UNLB (Southern Biotechnology Associates), Le^y-HSA (Alberta Research Council).

Methods: Dilute primary antibody or antigen to 1.0 µg/ml in 0.05M Carb/Bicarb buffer. Add 100µl of the diluted solution per well in Immulon 2 plates. Seal plates and incubate O.N. at 4°C.

- 5 Block plates by flicking them and blotting on paper towels. Add 200µl/well of Genetic Systems, Specimen Diluent Concentrate (1x). Incubate at least 1 hour at room temperature and then dump the contents of the plates. Wash the plates 3x in saline/Tween. Blot to dry. Allow the plates to dry at R.T. (45 min. to 1 hour). Seal and store the plates at 4°C.

10

Test samples as follows. Dilute samples and standards in Specimen Diluent at 1:10. Perform serial dilutions in separate round bottom plates. Transfer 100µl/well of final dilutions to antigen coated assay plates; then incubate O.N. at 4°C. Wash plates 3x with saline/Tween.

15

For conjugation add 100 µl/well of antibody-HRP conjugate in Genetic Systems Conjugate Diluent (1x). Incubate plates at Room Temp. for 60 min. Wash plates 3x in saline/Tween.

- 20 Add 100 µl/well of Genetic Systems EIA Chromogen Reagent (TMB) 1:100 in EIA Buffered Substrate (1x). Incubate at R.T. for 15 min. and stop with 1N H₂SO₄ 100 µl/well. Read plate at 450/630nm in EIA plate reader.

EXAMPLE 2

25

Construction of CH₂ deleted BR96 molecules

Strategy for Deleting CH₂ Domains: To construct CH₂ deleted BR96 molecules, the hinge, CH₂ and CH₃ domains were removed from chimeric BR96 and humanized

BR9696-2 IgG1 molecules by an Eco47-III restriction digestion in non-coding regions. The hinge and CH₃ domains were amplified by polymerase chain reaction (PCR) from a human IgG1 (pNγ1.14) molecule lacking the CH₂ domain. Two oligonucleotides (Sense 49mer, Antisense 50mer) homologous to the sequences of
 5 IgG1 constant region at both sides preserving E.co47-III sites were synthesized. The amplified hinge and CH₃ domain PCR fragments were added into Eco47-III sites on BR96 IgG1 molecules by in vivo homologous recombination (P. Bubeck et al., Nucleic Acid Research (1993) 21:3601-3602). The new BR96 IgG1 molecules were verified by restriction mapping and sequencing.

10

A sewing PCR strategy was used for the construction of CH₂ deleted human IgG1 (pNγ1.14) (Robert M. Horton, et al. (1990) Biotech 8 (5)P, 528).

The CH₁ domain was amplified as a 580 bp fragment with a sense oligonucleotide
 15 (5' TGG CAC CGA **AAG CTT** TCT GGG GCA GGC CAG GCC TGA 3') (primer A) and an antisense oligonucleotide (5' **TCC GAG CAT GTT GGT ACC CAC GTG GTG GTC GAC** GCT GAG CCT GGC TTC GAG CAG ACA 3') (primer B) from a linearized human IgG1 constant region vector (pNγ1.7). The PCR fragment extends from the 5' end of the Hind-III site (in bold) through the Cel-II, Sal-I, Dra-
 20 III, Kpn-I, 6 bp nucleotide spacer and Mro-I sites (in bold) at the 3' end of the CH₁ domain.

The CH₃ domain was then partially amplified (to the Xba-I site) with a sense primer
 25 (5' **GTC GAC CAC CAC GTG GGT ACC AAC ATG TCC GGA GCC ACA** TGG ACA GAG GCC GGC T 3') (primer C) and an antisense primer (5' CTG GTT CTT GTT CAT CTC CTC **TCT AGA TGG** 3') (primer D) from a linearized human IgG1 constant region vector (pNγ1.7). A PCR fragment (about 150 bp) with Sal-I, Dra-III, Kpn-I, 6 nucleotide spacer and Mro-I sites (in bold) on its 5' end, extends only through the Xba-1 site (in bold) within the CH₃ domain.

The CH₁ and CH₃ partial PCR fragments were combined in a PCR without any primer. The reaction was run through two full cycles of denaturation and re-annealing to allow the fragments to combine at the homologous region at the 3' ends. Primers A and D (described above) were added to the reaction and the PCR cycle was completed. The polymerase extends the DNA with primer A and primer D, yielding a full-length (660 bp) PCR fragment. The newly extended PCR fragment is arranged from the 5' end to the 3' end in the following order: Hind-III - CH₁ - Cel-II - Sal-I - Dra-III - Kpn-I - 6 bp spacer - Mro-I - CH₃ partial - Xba-I.

10

The combined PCR fragment, with the CH₁ and partial CH₃ domains, was then cloned by a blunt end ligation into a Sma-I site on a pEMBL18 vector and the sequence was confirmed by dideoxy sequencing (Sanger et al. (1977) PNAS (USA) 74:5463-5466).

15

To transfer the CH₁ and partial CH₃ into a mammalian expression vector, both the pEMBL18 and pNγ1.7 vectors were digested with Hind-III and Xba-I. The Hind-III and Xba-I fragment was ligated into the same sites on a linearized pNγ1.7 vector. The new construct, with CH₁ and a full CH₃ domain, was designated the pNγ1.10 vector.

20

The hinge fragment was amplified from a Hind-III digested pNγ1.7 vector with the primers designed to flank the hinge exon with a Sal-I and a Dra-III cloning site at each end. These sites also exist between the CH₁ and CH₃ domains of the pNγ1.10 construct. The sense oligonucleotide (5' ACC ATG **GTC GAC** CTC AGA CCT GCC AAG AGC CAT ATC 3') with a 6 bp spacer and a Sal-I cloning site (in bold) and the antisense oligonucleotide (5' CAT GGT **CAC GTG** GTG TGT CCC TGG ATG CAG GCT ACT CTA G 3') with a 6 bp spacer and a Dra-III cloning site (in bold) were used for the amplification of the hinge fragment (250 bp).

25

The hinge region PCR fragment was cloned into a Sma-I site on pEMBL18 by blunt end ligation. Both the pEMBL18 with the hinge domain and the pN γ 1.10 with the CH₂ and CH₃ domains were digested with Sal-I and Dra-III. The digested hinge
5 fragment was cloned into the Sal-I and Dra-III linearized sites on the pN γ 1.10 vector. The new construct, now carrying the CH₁, hinge and CH₃ domains, was designated pN γ 1.11.

To make the final CH₂ deleted human IgG1 construct, both the pN γ 1.11 construct
10 and pN γ 1.11 vector were digested with BamHI and HindIII. A fragment containing the CH₁, hinge and CH₃ domains was cloned into the linearized pN γ 1.11 vector. The new constant region IgG1 construct lacks the CH₂ domain and is designated pN γ 1.14 (Figure 11).

15 For digestion of BR96 IgG1 with Eco47-III, a restriction fragment with hinge, CH₂ and CH₃ domains was identified on the constant region sequence of BR96 IgG1 vector in both chimeric and humanized molecules. The 5' end of this fragment lies inside the intron between CH₁ and hinge and the 3' end is located inside the CH₃ intron of the BR96 IgG1 molecule. The hinge, CH₂ and CH₃ domains (1.368 kb
20 fragment) were removed from BR96 IgG1 molecules by Eco47-III restriction digestion. The Eco47-III is a blunt end cutter. The BR96 IgG1 DNA digested with this enzyme does not require any pretreatment before cloning. Figure 12 is a diagrammatic representation of the pD17-hBR96-2 vector showing the Eco47-III sites used in cloning.

25

The CH₂ deleted BR96 IgG1 was then constructed as follows. The hinge and CH₃ domains were amplified from a CH₂ deleted L6 IgG1 (pN γ 1.14) construct with a sense oligonucleotide (5'

CAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCGCTGACCTCAG

A 3') homologous to the constant region sequence of IgG1 at the 5' end of the Eco47-III site (in bold) and an antisense oligonucleotide

(5'GGAAAGAACCATCACAGTCTCGCAGGGG

CCCAGGGC**AGCGCTGGGTGCTT** 3') homologous to the constant region

- 5 sequence of IgG1 at the 3' end of the Eco47-III site (in bold). The Eco47-III site at the 3' end of the pN γ 1.14 construct is modified in the cloning process. The Eco47-III site is thus introduced into an antisense primer and used in amplification of the hinge and CH₃ domains.

- 10 The pD17-BR96 IgG1 vector was digested with Eco47-III and the hinge, CH₂ and CH₃ domains were removed. The linearized pD17-BR96 IgG1 vector was mixed with equimolar amounts of hinge and CH₃ PCR fragments. Cotransformation of the PCR fragment with linearized DNA into E.coli DH5a competent cells resulted in a recombinant molecule, mediated by homologous recombination in bacteria. This
- 15 construct lacks the CH₂ domain of BR96 IgG1 molecules, and is designated pD17-BR96-dCH2 (Figure 13).

1.9 grams of CH₂-deleted chimeric BR96 was obtained as raw material from 89L of culture supernatant.

20

EXAMPLE 3

Toxicity, localization and clearance of CH₂-deleted chimeric BR96 was tested in vivo as follows.

25

Three dogs received 400 mg/m² of cBR96-A, the CH₂ deletion mutant of chimeric BR96, and two received chimeric BR96. Both molecules had been mildly reduced and alkylated. This is required to prevent dimerization of the deletion mutant into a tetravalent form. Both control dogs experienced the typical GI toxicity and none of

the three receiving the mutant displayed any toxicity. The control dogs and two of the test dogs were sacrificed at 1 hr to obtain duodenal tissue to measure antibody localization. Both control dogs had grossly visible GI pathology, and the test dogs had normal appearing GI tissue. The third dog has continued to show no signs of toxicity.

Results: A significant amount of localization of the CH₂ deleted cBR96 (cBR96-A) occurred to the GI tract in dogs treated with 400 mg/m², although the intact chiBR96 localized slightly better. The levels of localization indicate that roughly equivalent amounts of intact and CH₂ deleted cBR96 was delivered to the GI tract in these dogs.

Table 5. Localization of cBR96 to GI tissue.

Group	Animal	Specific	mean
		Localization	
cBR96	#271	155	135
	#272	114	
cBR96-A	#273	126	89
	#274	52	

15

Using the mean level of specific localization, an amount of cBR96-A equivalent to at least 66% of the amount of cBR96 was delivered to the target organ of toxicity, the duodenum. Based on the dose ranging done with cBR96 in dogs (some clinical signs of toxicity seen at doses of 10 mg/m²), even if this difference is real, it could

not explain the difference between significant toxicity and no toxicity, evaluation to date indicated that dogs treated with cBR96-A had no toxicity, pending microscopic histopathologic examination. This evaluation was based on analysis of 2 frozen blocks per dog and 2 sections per block. Replicates were quite good. We also ran
5 historical frozen tissues from dogs treated with native cBR96 or F(ab)₂/BR96 and the levels of localization for those tissues were 110 and 0, respectively, consistent with our previous data.

Assuming that there is no toxicity at marginally higher (2X) doses of cBR96-A,
10 these data indicate that the CH₂ domain is associated with the induction of acute gastroenteropathy, and that the removal of this domain prevents the induction of gastroenteropathy mediated by BR96.

This study confirms the results showing that F(ab')₂ is not toxic in the dog model
15 and that the toxicity is mediated by the constant region. The CH₂ deletion mutant is a candidate for targeting agents clinically. Because of the very long half-life of chimeric BR96, some decrease in the mutant's half-life should be acceptable.

Figure 1 shows the measurement of the clearance of the cBR96-A in high Le^y
20 expressing dogs. The study used chimeric versus constant region mutant of cBR96-2.

CBR96-2 did clear faster than the chimeric BR96. The localization of cBR96-A to the gastrointestinal epithelium is not significantly affected by this more rapid
25 clearance. More than enough of the cBR96-A localized to have caused toxicity.

Discussion: The constant region of chimeric IgG is responsible for the GI toxicity seen in clinical trials, e.g. with chiBR96-dox. The GI toxicity seen in the dog model is very similar to the clinical toxicity. Both in man and dog, administration of the

unconjugated antibody mediates an acute GI toxicity characterized by rapid onset of vomiting, often with blood.

5 In man the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach. Although the pathology of the wound is limited and resolves, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity.

10 This toxicity is mediated in man and dog by the antibody molecule alone. At higher doses of the antibody-dox conjugate, additional toxicity is seen in the dog model, probably due to doxorubicin. Although the intact IgG of BR96 causes toxicity in dog and man, the F(ab')₂ molecule (divalent and lacking only in the constant region) is not toxic in dogs. This finding has motivated our attempts at high levels, and improves the affinity and specificity of BR96 for tumor antigen.

15 The CH₂ domain is known to mediate complement and FcR binding. It was not known that structural alteration of the CH₂ domain would result in immunoglobulin-induced toxicity inhibition.

20 Toxicology study of hBR96-2B

The toxicology study of hBR96-2B in high Lewis Y expressor dogs (n=2) showed that a dose of 400 mg/m² did not cause hematemesis nor bloody stools, in contrast to BR96 which consistently causes one or both signs. A dog sacrificed at 24 hrs had
25 normal gross appearance of the GI tract, again in marked contrast to chimeric BR96 which causes hemorrhagic lesions and mucosal erosions.

EXAMPLE 4

- The polymerase chain reaction (PCR) is a widely used and versatile technique for the amplification and subsequent modification of immunoglobulin genes. The rapidity and accuracy with which antibody genes can be modified in vitro has produced an assortment of novel antibody genes can be modified in vitro has produced an assortment of novel antibodies. For example, PCR methods have been used for engineering antibodies with increased affinity to antigen, for "humanizing" antibodies, and for modulating effector function (Marks, J.D., A.D. Griffiths, M. Malmqvist, T. Clackson, J.M. Bye and G. Winter. 1992. Bypassing immunization: high affinity human antibodies by chain shuffling. *Bio/Technology* 10:779-783; Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96 Fab. *J. Biol. Chem.* 271:22611-22618; Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. *Immunology.* 86:319-324).
- As part of a more comprehensive study, we desired to introduce various site specific mutations in the CH₂ constant domain of human IgG₁. Six specific amino acid residues distributed throughout the CH₂ domain previously identified to play a role in immune effector function were marked as targets for mutagenesis (Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH₂ domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. *Immunology.* 86:319-324; Duncan, A.R. and G. Winter. 1988. The binding site for C1q on IgG. *Nature* 332:738-740; Tao, M.-H., R.I.F. Smith and S.L. Morrison. 1993. Structural features of human immunoglobulin G that determine isotype-specific differences in complement

activation. J.Exp.Med. 178:661-667). five of the six residues were grouped into two clusters-one cluster consisting of two residues, two amino acids apart (Location 1, or L1); and a second cluster consisting of three residues spanning a sequence of five amino acids (L2). The remaining amino acid position (L3) made for the total of six
5 residues. We were interested in constructing a panel of mutant CH₂ domain IgGs consisting of each L mutation by itself as well as in combination with other L mutants (e.g., L1; L1; and L2; L1, L2 and L3; etc.).

Various *in vitro* methods have been described where PCR is used to simultaneously
10 introduce distally located site-specific mutations within a gene sequence (Ho, S.N., H.D. Hunt, R.M. Horton, J.K. Pullen and L.R. Pease. 1989. Site-directed mutagenesis by overlap extension. Gene 77:51-59; Ge, L. and P. Rudolph. 1996. Simultaneous introduction of multiple mutations using overlap extension PCR. BioTechniques 22:28-30). Alternatively, an *in vivo* procedure termed recombination
15 PCR (RPCR) has also successfully been used for rapidly and efficiently generating distally located site-specific mutations (Jones, D.H. and S.C. Winistorfer. 1993. Use of polymerase chain reaction for making recombinant constructs. p.241-250. In B.A. White (Ed.), Methods in Molecular Biology, Vol. 15. Humana Press Inc., Totowa, NJ, Jones, D.H. And B.H. Howard. 1991. A rapid method for
20 recombination and site-specific mutagenesis by placing homologous ends on DNA using polymerase chain reaction. BioTechniques 10:62-66). RPCR uses *E. Coli*'s recombination machinery to generate intact circular recombinant plasmids from a transfected mixture of linear PCR-generated product and linearized vector. *In vivo* recombination is mediated through the joining of nucleotide sequences designed into
25 the 5' ends of both PCR primers that are homologous to DNA sequences encoded by the vector. In this report we describe an extension of the RPCR procedure for simultaneously introducing complex combinations of mutations into an antibody CH₂ domain.

- Humanized BR96 variable region heavy and light chain genes, previously cloned and co-expressed as an assembled active Fab fragment in an M13 phage expression vector, provided the starting material (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96 Fab. J. Biol. Chem. 271:22611-22618). The heavy and light chain V genes were amplified by PCR from a single-stranded M13 DNA template and subcloned by *in vivo* recombination (Jones, D.H. And B.H. Howard. 1991. A rapid method for recombination and site-specific mutagenesis by placing homologous ends on DNA using polymerase chain reaction. BioTechniques 10:62-66) into vectors pD17-hG1a and pD16-hCκ, to form pBR96-hG1a and pBR96-hCκ respectively. pD17-hG1a and pD16-hCκ are eukaryotic immunoglobulin expression vectors derived from pcDNA3 (Invitrogen, San Diego, CA). The plasmid pBR96-hG1a was further modified by site-directed mutagenesis to introduce two Eco47-III restriction sites flanking the immunoglobulin hinge-CH₂-CH₃ domains using standard procedures. The recipient vector was then prepared by digesting pBR96-hG1a with Eco47-III, isolating the vector backbone by agarose gel electrophoresis followed by extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA).
- The strategy for introducing multiple mutations within the immunoglobulin CH₂ gene, shown in Figure 24, relies on the *in vivo* homologous recombination of several independently amplified PCR products with each other as well as with the pBR96-hG1a vector DNA. For introducing mutations at two distal locations two PCR products are synthesized (Figure 24B). One end of each PCR product is for recombining with an homologous end of the linear vector, and the other end, encoding the mutation(s) of interest, is for recombining with the neighboring PCR product. As shown in Figure 24B, additional distally-located mutations can be introduced into a target sequence by increasing the number of PCR products

proportionately. The recombination of neighboring PCR products always occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends (e.g., A1, A2) contain complementary mutant residues.

The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers (Rs and Ra) contain sequences for recombining with the end regions of the Eco47-III digested pBR96-hG1a vector.

- Each L mutation was amplified in a separate PCR reaction. The reaction conditions were 250 ng intact pBR96-hG1a DNA template, 10 ul of 1X *Pfu* buffer (Stratagene, Inc. San Diego, CA), 10 nmol dNTPs, 200ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase in a 100ul reaction volume. Samples were first denatured at 95° C for 5 min, cooled to 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, followed by a final extension at 72°C for 7 min in a Perkin-Elmer DNA Thermal Cycler (Norwalk, CT). The amplified products were purified from a 1% agarose gel, extracted with Qiagen Gel Extraction kit and the recovered DNA quantitated. 50 ng of each PCR product was mixed with 25 ng of the Eco47-III digested pBR96-hG1a vector, transfected into Max competent *E. coli* DH5α according to the manufacturer's procedure (GIBCO BRL/Life Technologies, Gaithersburg, MD), and the entire transfection reaction plated onto selective LB agar plates containing 100 ug/ml ampicillin.

The results of several cloning experiments are summarized in the Table that follows. Typically the transformations produced from 80 to 200 bacterial colonies. Individual colonies were selected and grown overnight in 2 ml liquid cultures for isolation of miniprep plasmid DNA (Qiagen) and analysis by Eco47-III restriction

endonuclease mapping. Among 24 independent transformants analyzed from triple homologous recombination events (two PCR products plus vector) 11 clones contained the predicted 1.4 kpb DNA insert.

- 5 Figure 25 shows a sample diagnostic restriction analysis of DNA prepared from clones derived from quadruple homologous recombination events (three PCR products plus vector). Additional sampling of clones resulting from quadruple recombination yielded a cloning efficiency of 29% (7 clones containing inserts/24 clones sampled). At this point, due to the small sampling sizes, we do not know
10 whether the differences in the cloning efficiencies observed between the triple and quadruple recombination events are meaningful.

- To evaluate the expression of Le γ -binding activity of the CH₂ mutant IgGs, miniprep DNAs from 6 clones derived from the triple recombination reaction and 6
15 clones derived from the quadruple recombination reaction exhibiting the predicted diagnostic Eco47-III restriction patterns were isolated, mixed with pBR96- hC κ DNA and used to co-transfect COS7 cells. 48 hour spent supernatants from 3 ml cultures were assayed for total IgG production and for Le γ binding activity by enzyme-linked immunosorbent assay (EIA) as described (Yelton, D.E., M.J. Rosok,
20 G.A. Cruz, W.L. Cosand, J. Bajorath, I. Hellstrom, K.-E. Hellstorm, W.D. Huse and S.M. Glaser. 1995. Affinity maturation of the BR96 anti-carcinoma antibody by codon-based mutagenesis. J.Immunol. 155:1994-2004). All twelve cultures were found to secrete approximately 2-3 ug/ml Le γ -reactive IgG. The spectrum of Le γ binding activities were all similar to that of native humanized BR96 IgG indicating
25 that the homologously recombined antibodies did not acquire any gross mutations that could affect antigen binding. To confirm that the desired CH₂ mutations had been incorporated, and to evaluate the recombined genes for misincorporated nucleotides, four of the clones producing functional antibody were sequenced using Sequenase Version 2 DNA Sequencing Kit (United States Biochemical). One clone

was found to contain a single nucleotide change within the forward PCR primer used for mediating recombination with vector DNA. We are uncertain whether this error occurred during chemical synthesis of the oligonucleotide primer or is a result of misincorporation during the PCR reaction, despite the fact that we used a

5 thermostable polymerase with proofreading activity.

A RPCR procedure for homologously recombining up to three separate PCR-generated mutated antibody sequence products into a eukaryotic expression vector for the rapid construction of engineered IgG molecules is described herein. The

10 advantage of this approach is the ability to simultaneously introduce multiple distally-located mutations with PCR products synthesized by a single round of PCR. Recombinant DNAs are produced with a reasonably high cloning efficiency and fidelity of correct nucleotide sequences. The ability to efficiently rejoin several distinct PCR products should permit combinatorial strategies for constructing

15 complexly mutated protein domains as well as broadening the number and location of desired mutations.

Analysis of transformants generated by multiple-fragment RPCR.

Mutant IgGs Constructed	PCR Fragments in reaction	HR ^a events	Colonies Analyzed	Cloning Efficiency ^b
2	2	triple	24	45%
2	3	quadruple	24	33%
^a HR-homologous recombination				
^b Cloning efficiency (number of clones containing 1.4kbp insert/total number of colonies)				

EXAMPLE 5

This example provides two methods for introducing site specific mutations into the
5 CH2 domain of human IgG1 constant region containing vectors.

One method involves PCR amplification of a segment or segments of the constant region, wherein mutations are introduced using appropriately constructed oligonucleotides. The vector receiving the fragment(s) is digested with a restriction
10 enzyme to linearize the vector. PCR amplification primers are designed so that the 5' ends of the PCR fragments can hybridize to the DNA sequence of the vectors. If more than one PCR fragment is amplified, then common sequences to the two fragments are introduced by oligonucleotides. Bacteria are transfected with the PCR fragments and with the digested vector. The fragments and vector can recombine by
15 homologous recombination using the bacteria's recombination machinery. Bacterial colonies are selected and the DNA is analyzed by size and restriction map as a preliminary determination that the vector and fragment(s) recombined correctly. Correct insertion of fragments with the mutations is confirmed by dideoxynucleotide sequence analysis. DNA is then introduced into mammalian cells as described for
20 the CH2 deleted antibody, and the expressed antibody analyzed for binding and functional activity.

By way of example, mutations Leu to Ala at residue 235 in CH2 and Gly to Ala at residue 237 were introduced by the procedure disclosed in Example 4. The heavy
25 chain vector used for this procedure was pD17-hG1a, similar to pD17-BR96 vector described herein except that humanized V regions (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K-E. Hellstrom, I, Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse, and S.M. Glaser, 1996. J. Biol. Chem 271 37:22611-22618) with three affinity mutations (H1, H2, and H3 mutations) were substituted.

pBR96-hG1a contains two *Eco47-III* restriction sites flanking the Ig hinge-CH2-CH3 domains. The recipient vector was prepared by (1) digesting pBR96-hG1a with *Eco47-III*, (2) isolating the vector by agarose gel electrophoresis, and (3) extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA). To introduce mutations at a single location, such as for positions 235 and 237, two PCR products were synthesized.

To introduce two distally located mutations, such as for mutant F (also referred to herein as hBR96-2F) with mutations at 235, 237, 331, requires 3 PCR products. The recombination of neighboring PCR products occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends contain complementary mutant residues. The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers containing sequences for recombining with the end regions of the *Eco47-III* digested pBR96-hG1a vector.

PCR amplification used 250 ng intact pBR96-hG1a DNA template, 10 μ l of 10X *Pfu* buffer (Stratagene, Inc., San Diego, CA), 10 nmol dNTPs, 200 ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase (Stratagen, Inc. San Diego, CA) in 100 μ l reaction. Samples were denatured at 95°C for 5 min, annealed at 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, and a final extension at 72°C for 7 min. The amplified products were purified from a 1% agarose gel, extracted with the Qiagen Gel Extraction kit and quantitated. 50 mg of each PCR product was mixed with 25 ng of the *Eco47-III* digested pBR96-hG1a vector and transfected in E.coli MAX Efficiency DH5 α TM according to the

manufacturer's instructions (GIBCO BRL/Life Technologies, Gaithersburg, MD).

The entire transfection reaction was plated onto LB agar plated containing 100 µg/ml ampicillin.

5 Bacterial colonies were selected and grown overnight at 37° C in 2 ml liquid cultures. DNA was isolated and analyzed by Eco47-III restriction endonuclease mapping. Clones with the correct size insert were sequenced (Sequenase Version 2, U.S. Biochemical Corp., Cleveland, OH).

10 The second method for introducing site specific mutations into the CH₂ domain of human IgG1 involved the method of Kunkel (1987 Methods Enzymology, supra). For this procedure pD17-hG1b DNA with the F1 origin of replication was introduced into electrocompetent E. coli CJ236 dut-ung- (Bio-Rad Laboratories, Hercules, CA) by electroporation according to manufacturer's instructions. PD17-
15 hG1b is a vector having a constant region but no variable region. The F1 ori site allows treatment of this vector as a phagemid.

Bacteria containing the plasmid were selected by ampicillin resistance. Single stranded uridinylated DNA was prepared using the Muta-Gene Phagemid In Vitro

20 Mutagenesis Version 2 protocol (Bio-Rad). Mutations were introduced by site-directed mutagenesis with the appropriate antisense oligonucleotide. For molecules with mutations at more than one location, mutations were introduced by either of the two methods discussed above. One method would be to (1) prepare one mutant, for example, mutant 2C (also referred to herein as BR96-2C) with the mutations at
25 residues 318, 320, 322, (2) isolate ssDNA, and (3) introduce a second mutation set with the appropriate anti-sense oligonucleotide. The second method would be to anneal two antisense oligonucleotides with the same uridinylated ssDNA and screen for mutants with both sets of changes. Mutant 2H (hBR96-2H) was also prepared by a combination of these methods.

The V region of humanized BR96-2 heavy chain was introduced by the homologous recombination method described above in pD17-hJm14.H1. The pD17-hJm14.H1 plasmid contains the BR96 humanized variable region with the H1/H2/H3

- 5 mutations and the plasmid was used to transfect mutant sequences into mammalian cells. The pD17G1b vector containing the Fc mutation(s) was digested with NheI for 3 hr at 37° C and the DNA isolated by methods described above. Insertion of the V region into the vector was determined by size and restriction enzyme mapping and confirmed by sequence analysis.

10

Transient expression of whole antibodies was performed by transfection of COS cells. For production of antibody, stable transfections of CHO cells were performed (see description of deleted CH2 mutant). All mutants were purified from CHO culture supernatants by protein A chromatography.

15

The oligonucleotide primers homologous to the vector and used to introduce the constant regions mutations were as follows:

Oligonucleotides homologous to vector sequences:

Sens(sense)CH2 E47-3-5: CAG GGA GGG AGG GTG TCT GCT GGA AGC

20 CAG GCT CAG CGC TGA CCT CAGA

D CH2 E47-3 A (antisense): GGA AAG AAC CAT CAC AGT CTC GCA GGG
GCC CAG GGC AGC GCT GGG TGC TT

Oligonucleotides to mutate Leu235 to Ala and Gly237 to Ala (underlined sequences

25 show sites of mutation):

Antisense CH2 L235-G237/aa: GAA GAG GAA GAC TGA CGG TGC CCC
CGC GAG TTC AGG TGC TGA GG

SensCH2 L235-G237/AA: CCT CAG CAC CTG AAC TCG CGG GGG CAC
CGT CAG TCT TCC TCT TC

Oligonucleotides to mutate Glu318, Lys320, Lys322 to Ser

Antis(antisense)CH2 EKK/SSS-2: CTG GGA GGG CTT TGT TGG AGA CCG
AGC ACG AGT ACG ACT TGC CAT TCA GCC

5 Oligonucleotides to mutate Pro331 to Ala:

Antis CH2 P331/A/3: GAT GGT TTT CTC GAT GGC GGC TGG GAG GGC

Sense CH2 P33/A: GCC CTC CCA GCC GCC ATC GAG AAA ACC ATC

Alternative antisense oligo to introduce Ala at 331 by site-directed mutation:

CH2P331A: GAT GGT TTT CTC GAT AGC GGC TGG GAG GGC TTT G

10

Oligonucleotides to mutate Glu318 to Ser, Lys320 to Ser, Lys322 to Ser, and Pro331 to Ala:

Antis CH2 EKKP/SSA-6: GAT GGT TTT CTC GAT GGC GGC TGG GAG
 GGC TTT GTT GGA GAC CGA GCA CGA GTA CGA CTT GCC ATT CAG

15 CCA GTC CTG GTG

Sense CH2 EKKP/SSA-6: CAC CAG GAC TGG CTG AAT GGC AAG TCG
 TAC TCG TGC TCG GTC TCC AAC AAA GCC CTC CCA GCC GCC ATC
 GAG AAA ACC ATC

20

In vitro Assays of the Mutants

Results of the CDC demonstrate that mutant hBR96-2B has approximately 10 fold less activity than the control hBR96-1 (two affinity mutations, one in H2 and one in H3, refer to previous patent (Figure 20)). The mutants that have the least ability to kill cells in the presence of complement is hBR96-2C with the triple mutations at positions 318, 320, and 322 and the hBR96-2H mutant (least cytotoxic antibodies in the panel) which contains all six mutations at the three different locations. ADCC activity was most affected by the CH2 deleted hBR96-2 molecule (Figure 21).

hBR96-2B and -2H lost between 100 and 1000 fold activity to kill in the presence of effector cells. In the ADCC assay the hBR96-2B molecule also lost approximately 10 fold activity (Figure 21).

- 5 Figures 26-28 provide the amino acid sequences for the heavy chain variable region for both chimeric and humanized BR96 having the H1, H2, and H3 mutations. The amino acid sequence for the light chain variable region is known and methods for generating it are found in PCT Application No. 95/305444. Additionally provided is the amino acid sequence for the IgG1 constant region. Mutations in the constant
- 10 region are marked.

SEQUENCE LISTING

- 5 (1) GENERAL INFORMATION
- (i) APPLICANT: Bristol-Myers Squibb Co.
- (ii) TITLE OF THE INVENTION:
10 A METHOD FOR INHIBITING
IMMUNOGLOBULIN-INDUCED TOXICITY FROM THE USE OF
IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS
- (iii) NUMBER OF SEQUENCES: 13
- 15 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Merchant & Gould
(B) STREET: 11150 Santa Monica Blvd., Suite 400
(C) CITY: Los Angeles
(D) STATE: CA
20 (E) COUNTRY: USA
(F) ZIP: 90025
- (v) COMPUTER READABLE FORM:
25 (A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 2.0
- (vi) CURRENT APPLICATION DATA:
30 (A) APPLICATION NUMBER: PCT/US97/_____
(B) FILING DATE: 01-AUG-1997
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
35 (A) APPLICATION NUMBER: 60/023,033
(B) FILING DATE: 02-AUG-1996
- 40 (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Adriano, Sarah B
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(C) REFERENCE/DOCKET NUMBER: 30436.43WOU1
- 45 (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 310-445-1140
(B) TELEFAX: 310-445-9031
(C) TELEX:
- 50 (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
55 (A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5 TGGCACCAGAA AGCTTTCTGG GGCAGGCCAG GCCTGA 36

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

20 TCCGGACATG TTGGTACCCA CGTGGTGGTC GACGCTGAGC CTGGCTTCGA GCAGACA 57

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCGACCACC ACGTGGGTAC CAACATGTCC GGAGCCACAT GGACAGAGGC CGGCT 55

35 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTGGTTCTTG TTCATCTCCT CTCTAGATGG 30

(2) INFORMATION FOR SEQ ID NO:5:

50

(i) SEQUENCE CHARACTERISTICS:

- 55 (A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACCATGGTCG ACCTCAGACC TGCCAAGAGC CATATC 36

(2) INFORMATION FOR SEQ ID NO:6:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

15 CATGGTCACG TGGTGTGTCC CTGGATGCAG GCTACTCTA 39

(2) INFORMATION FOR SEQ ID NO:7:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 49 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

30 CAGGGAGGGA GGGTGTCTGC TGGAAGCCAG GCTCAGCGCT GACCTCAGA 49

(2) INFORMATION FOR SEQ ID NO:8:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 50 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

45 GGAAAGAACC ATCACAGTCT CGCAGGGGCC CAGGGCAGCG CTGGGTGCTT 50

(2) INFORMATION FOR SEQ ID NO:9:

50 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8691 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCCG GCTTCGAATA GCCAGAGTAA 60

CCTTTTTTTT TAATTTTATT TTAATTTTATT TTTGAGATGG AGTTTGGCGC CGATCTCCCG 120

	ATCCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGC	GAGCAAAATT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGTTTTGCGC	300
	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAAT	360
5	AGTAATCAAT	TACGGGGTCA	TTAGTTCATA	GCCCATATAT	GGAGTTCCGC	GTTACATAAC	420
	TTACGGTAAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGTCAATAA	480
	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTTACGGTA	AACTGCCCAC	TTGGCAGTAG	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCC	CCTGGCATTG	TGCCCAGTAC	ATGACCTTAT	660
10	GGGACTTTTC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	720
	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	960
15	TTAATACGAC	TCATATAGG	GAGACCCAAG	CTTGGTACCA	ATTAAATTG	ATATCTCCTT	1020
	AGGCTCTGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGCGGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCTT	TGTTTTAAAA	GGTGTCCAGT	1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
20	TCTCCTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATTA	CATGTATTGG	GTTCGCCAGA	1260
	CTCCAGAGAA	GAGGCTGGAG	TGGGTCGCAT	ACATTAGTCA	AGGTGGTGAT	ATAACCGACT	1320
	ATCCAGACAC	TGTAAAGGGT	CGATTCAACA	TCTCCAGAGA	CAATGCCAAG	AACACCTGT	1380
	ACCTGCAAAAT	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500
	CTAGCACCAA	GGGCCCATCG	GTCTTCCCCC	TGGCACCCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
25	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCGGTG	ACGGTGTCTG	1620
	GGAACTCAGG	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCTTA	CAGTCTCTAG	1680
	GACTCTACTC	CCTCAGCAGC	GTGGTCACCG	TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCT	1740
	ACATCTGCAA	CGTGAATCAC	AAGCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTTGGTGAGA	1800
	GGCCAGCACCA	GGGAGGGAGG	GTGTCTGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCTGGACG	1860
30	CATCCCGGCT	ATGCAGCCCC	AGTCCAGGGC	AGCAAGGCAG	GCCCCGTCTG	CCTCTTCACC	1920
	CGGAGGCCTC	TGCCCCCCCC	ACTCATGCTC	AGGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG	1980
	GCTCTGGGCA	GGCACAGGCT	AGGTGCCCCCT	AACCCAGGCC	CTGCACACAA	AGGGGACAGT	2040
	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC	CGGGAGGACC	CTGCCCCCTGA	CCTAAGCCCA	2100
	CCCCAAAGGC	CAAACCTCTC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA	2160
35	GTAACCTCCA	ATCTTCTCTC	TGCAGAGCCC	AAATCTTGTG	ACAAAACCTCA	CACATGCCCA	2220
	CCGTGCCCCAG	GTAAGCCAGC	CCAGGCCTCG	CCCTCCAGCT	CAAGGCGGGA	CAGGTGCCCT	2280
	AGAGTAGCCT	GCATCCAGGG	ACAGGCCCCA	GCCGGGTGCT	GACACGTCCA	CCTCCATCTC	2340
	TTCCCTCAGCA	CCTGAACCTC	TGGGGGGACC	GTCACTCTTC	CTCTTCCCCC	CAAAACCCAA	2400
	GGACACCCTC	ATGATCTCCC	GGACCCCTGA	GGTCACATGC	GTGGTGGTGG	ACGTGAGCCA	2460
40	CGAAGACCCT	GAGGTCAAGT	TCAACTGGTA	CGTGAGCGGC	GTGGAGGTGC	ATAATGCCAA	2520
	GACAAAGCCG	CGGGAGGAGC	AGTACAACAG	CACGTACCGT	GTGGTCAGCG	TCCTCACCGT	2580
	CCTGCACCAG	GACTGGCTGA	ATGGCAAGGA	GTACAAGTGC	AAGGTCTCCA	ACAAAGCCCT	2640
	CCCAGCCCCC	ATCGAGAAAA	CCATCTCCAA	AGCCAAAGGT	GGGACCCGTG	GGGTGCGAGG	2700
	GCCACATGGA	CAGAGGCCGG	CTCGGCCAC	CCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	2760
45	CCTCTGTCCC	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA	CACCCTGCCC	CCATCCCGGG	2820
	ATGAGCTGAC	CAAGAACCAG	GTGAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCAGCG	2880
	ACATCGCCGT	GGAGTGGGAG	AGCAATGGGC	AGCCGAGAGAA	CAACTACAAG	ACCACGCCCTC	2940
	CCGTGCTGGA	TCCCGACGGC	TCCTTCTTCC	TCTACAGCAA	GCTCACCGTG	GACAAGAGCA	3000
	GGTGGCAGCA	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	3060
50	ACACGCAGAA	GAGCCTCTCC	CTGTCTCCGG	GTAAATGAGT	GCGACGGCCG	GCAAGCCCCC	3120
	GCTCCCCGGG	CTCTCGCGGT	CGCACGAGGA	TGCTTGGCAC	GTACCCCTTG	TACATACTTC	3180
	CCGGGCGCCC	AGCATGGAAA	TAAAGCACCC	AGCGCTGCCC	TGGGCCCCCTG	CGAGACTGTG	3240
	ATGGTTCTTT	CCACGGGTCA	GGCCGAGTCT	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	3300
55	GGGTCCCACT	GTCCCCACAC	TGGCCGAGGC	TGTGCAGGTG	TGCTGGGCC	CCCTAGGGTG	3360
	GGGCTCAGCC	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	3420
	AGCAGCACCT	GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCTGGG	GACAGACACA	3480
	CAGCCCCTGC	CTCTGTAGGA	GACTGTCTCTG	TTCTGTGAGC	GCCCCGTGTC	TCCCGACCTC	3540
	CATGCCCACT	CGGGGGCATG	CCTAGTCCAT	GTGCGTAGGG	ACAGGCCCTC	CCTCACCCAT	3600
	CTACCCCCAC	GGCACTAACC	CCTGGCTGCC	CTGCCAGGCC	TCGCACCCGC	ATGGGGACAC	3660

	AACCGACTCC	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GACTGGTGCA	GATGCCCACA	3720
	CACACACTCA	GCCCAGACCC	GTTCACAAA	CCCCGACTG	AGGTTGGCCG	GCCACACGGC	3780
	CACCACACAC	ACACGTGCAC	GCCTCACACA	CGGAGCCTCA	CCCGGGCGAA	CTGCACAGCA	3840
	CCCAGACCAG	AGCAAGGTCC	TCGCACACGT	GAACACTCCT	CGGACACAGG	CCCCACGAG	3900
5	CCCCACGCGG	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	3960
	TCAGACAAAC	CCAGCCCTCC	TCTCACAAGG	GTGCCCCTGC	AGCCGCCACA	CACACACAGG	4020
	GGATCACACA	CCACGTCACG	TCCCTGGCCC	TGGCCCACTT	CCCAGTGCCG	CCCTTCCCTG	4080
	CAGGACGGAT	CAGCCTCGAC	TGTGCCTTCT	AGTTGCCAGC	CATCTGTTGT	TTGCCCCTCC	4140
	CCCGTGCCCT	CCTTGACCCT	GGAAGGTGCC	ACTCCCCTG	TCCTTTCCTA	ATAAAATGAG	4200
10	GAAATTGCAT	CGCATTGTCT	GAGTAGGTGT	CATTCTATTG	TGGGGGGTGG	GGTGGGGCAG	4260
	GACAGCAAGG	GGGAGGATTG	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	4320
	ATGGCTTCTG	AGGCGGAAAG	AACCAGCTGG	GGCTCTAGGG	GGTATCCCCA	CGCGCCTGT	4380
	AGCGGCGCAT	TAAGCGCGGC	GGGTGTGGTG	GTTACGCGCA	GCGTGACCGC	TACACTTGCC	4440
	AGCGCCCTAG	CGCCCGCTCC	TTTCGCTTTC	TTCCCTTCCT	TTCTCGCCAC	GTTCGCCGGG	4500
15	CCTCTCAAAA	AAGGGAAAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	4560
	CTAACTCCGC	CCATCCCGCC	CCTAACTCCG	CCCATTCCG	CCCATTCTCC	GCCCCATGGC	4620
	TGACTAATTT	TTTTTATTTA	TGCAGAGGCC	GAGGCCGCT	CGGCCTCTGA	GCTATTCCAG	4680
	AAGTAGTGAG	GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	4740
	GCTGCGATTT	CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	4800
20	CCCGCTGCCA	TCATGGTTTC	ACCATTGAAC	TGCATCGTCG	CCGTGTCCCA	AAATATGGGG	4860
	ATTGGCAAGA	ACGGAGACCT	ACCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	4920
	AGAATGACCA	CAACCTCTTC	AGTGGAAGGT	AAACAGAAATC	TGGTGATTAT	GGGTAGGAAA	4980
	ACCTGGTTCT	CCATTCTCTG	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	5040
	AGTAGAGAAC	TCAAAGAACC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG	TTTGGATGAT	5100
25	GCCTTAAGAC	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	5160
	GGAGGCAGTT	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	5220
	ACAAGGATCA	TGCAGGAATT	TGAAAGTGAC	ACGTTTTTCC	CAGAAATTGA	TTTGGGGAAA	5280
	TATAAACTTC	TCCCAGAATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	5340
	AAGTATAAGT	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	5400
30	GCTCCCCTCC	TAAAGCTATG	CATTTTTATA	AGACCATGGG	ACTTTTGCTG	GCTTTAGATC	5460
	TCTTTGTGAA	GGAACCTTAC	TTCTGTGGTG	TGACATAATT	GGACAAACTA	CCTACAGAGA	5520
	TTTAAAGCTC	TAAGGTAAAT	ATAAAATTTT	TAAGTGTATA	ATGTGTTAAA	CTACTGATTC	5580
	TAATTGTTTG	TGTATTTTAG	ATTCCAACCT	ATGGAAGTGA	TGAATGGGAG	CAGTGGTGGA	5640
	ATGCCTTTAA	TGAGGAAAAC	CTGTTTTGCT	CAGAAGAAAT	GCCATCTAGT	GATGATGAGG	5700
35	CTACTGCTGA	CTCTCAACAT	TCTACTCTC	CAAAAAAGAA	GAGAAAGGTA	GAAGACCCCA	5760
	AGGACTTTCC	TTCAGAATTG	CTAAGTTTTT	TGAGTCATGC	TGTGTTTAGT	AATAGAAGTC	5820
	TTGCTTGCTT	TGCTATTTAC	ACCACAAAGG	AAAAAGCTGC	ACTGCTATAC	AAGAAAATTA	5880
	TGGAAAAATA	TTCTGTAACC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	5940
40	TTTTTCTTAC	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACATGCT	CAAAAATTGT	6000
	GTACCTTTAG	CTTTTTAATT	TGTAAAGGGG	TTAATAAGGA	ATATTGTAGT	TATAGTGCCCT	6060
	TGACTAGAGA	TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTTACTTGC	TTTAAAAAAC	6120
	CTCCCACACC	TCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	6180
	TTTATTGCAG	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	6240
	GCATTTTTTT	CACTGCATTC	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	6300
45	GTCTGGATCG	GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCAC	6360
	CCCAACTTGT	TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTT	6420
	ACAAATAAAG	CATTTTTTTC	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	6480
	TCTTATCATG	TCTGTATACC	GTGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	6540
	CTGTTTCCTG	TGTGAAATTG	TTATCCGCTC	ACAATTCAC	ACAACATACG	AGCCGGAAGC	6600
50	ATAAAGTGTA	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	6660
	TCACTGCCCC	CTTTCCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	6720
	CGCGCGGGGA	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCCG	CTTCTCTGCT	CCTGACTCG	6780
	CTGCGCTCGG	TCGTTCCGCT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	6840
	TTATCCACAG	AATCAGGGGA	TAACGAGGGA	AAGAACATGT	GAGCAAAGG	CCAGCAAAG	6900
55	GCCAGGAACC	GTA AAAAGGC	CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	6960
	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	7020
	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	7080
	ACCGGATACC	TGTCCGCCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTCTCTA	ATGCTACGC	7140
	TGTAGGTATC	TCAGTTCCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	7200

	CCCCGTTGAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	7260
	AGACACGACT	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	7320
	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	7380
	GTATTTGGTA	TCTGCGTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	7440
5	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	7500
	ACGCGCAGAA	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	7560
	CAGTGGAACG	AAAACCTCAC	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	7620
	ACCTAGATCC	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	7680
	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	7740
10	TTTCGTTTCAT	CCATAGTTGC	CTGACTCCCC	GTGCTGTAGA	TAACCTACGAT	ACGGGAGGGC	7800
	TTACCATCTG	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	7860
	TTATCAGCAA	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	7920
	TCCGCCTCCA	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCGCCAGTT	7980
	AATAGTTTGC	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACG	CTCGTCGTTT	8040
15	GGTATGGCTT	CATTACAGTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	8100
	TTGTGCAAAA	AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTCAGAAG	TAAGTTGGCC	8160
	GCAGTGTAT	CATCTATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	8220
	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	8280
	CGGCGACCGA	GTTGCTCTTG	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	8340
20	ACTTTAAAG	TGCTCATCAT	TGGAAGACGT	TCTTCGGGGC	GAAACTCTC	AAGGATCTTA	8400
	CCGCTGTTGA	GATCCAGTTC	GATGTAACCC	ACTCGTGAC	CCAACCTGATC	TTCAGCATCT	8460
	TTTACTTTCA	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAG	8520
	GGAATAAGGG	CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTCA	ATATTATTGA	8580
	AGCATTATATC	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	8640
25	AAACAAATAG	GGGTTCCGCG	CACATTTCCC	CGAAAAGTGC	CACCTGACGT	C	8691

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 8327 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

	GACGGATCGG	GAGATCTGCT	AGGTGACCTG	AGGCGCGCCG	GCTTCGAATA	GCCAGAGTAA	60
40	CCTTTTTTTT	TAATTTTATT	TTATTTTATT	TTTGAGATGG	AGTTTGGCGC	CGATCTCCCG	120
	ATCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGCGC	GAGCAAAAT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGTTTTGCGC	300
	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	CTTGACATTG	ATTATTGACT	AGTTATTAAT	360
45	AGTAATCAAT	TACGGGGTCA	TTAGTTCATA	GCCCCATATAT	GGAGTTCGCG	GTTACATAAC	420
	TTACGGTAAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGTCAATAA	480
	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTTACGGTA	AACTGCCCAC	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCC	CCTGGCATTG	TGCCCAGTAC	ATGACCTTAT	660
50	GGGACTTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	720
	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	960
55	TTAATACGAC	TCACTATAGG	GAGACCCAAG	CTTGGTACCA	ATTTAAATTG	ATATCTCCTT	1020
	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGCGGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCC	TGTTTTAAAA	GGTGTCAGT	1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
	TCTCCTGTGT	AACCTCTGGA	TTCATTTTCA	GTGACTATTA	CATGTATTGG	GTTCCGCCAGA	1260

	CTCCAGAGAA	GAGGCTGGAG	TGGGTGCGAT	ACATTAGTCA	AGGTGGTGAT	ATAACCGACT	1320
	ATCCAGACAC	TGTAAAGGGT	CGATTACCA	TCTCCAGAGA	CAATGCCAAG	AACACCCTGT	1380
	ACCTGCAAT	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500
5	CTAGCACAA	GGGCCCATCG	GTCTTCCCC	TGGCACCCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCGGTG	ACGGTGTCTG	1620
	GGAACTCAGG	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCCTA	CAGTCTCAG	1680
	GACTCTACTC	CCTCAGCAGC	GTGGTCAACG	TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCT	1740
	ACATCTGCAA	CGTGAATCAC	AAGCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTTGGTGAGA	1800
10	GGCCAGCACA	GGGAGGGAGG	GTGTCTGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCTGGACG	1860
	CATCCCGGCT	ATGCAGCCCC	AGTCCAGGGC	AGCAAGGCAG	GCCCCGTCTG	CCTCTTCACC	1920
	CGGAGGCCTC	TGCCCGCCCC	ACTCATGCTC	AGGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG	1980
	GCTCTGGGCA	GGCAGAGGCT	AGGTGCCCT	AACCCAGGCC	CTGCACACAA	AGGGGCAGGT	2040
	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC	CGGGAGGACC	CTGCCCCGA	CCTAAGCCCA	2100
15	CCCCAAAGGC	CAAACTCTCC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA	2160
	GTAACTCCCA	ATCTTCTCTC	TGCAGAGCCC	AAATCTTGTC	ACAAAATCA	CACATGCCCA	2220
	CCGTGCCCAG	GTAAGCCAGC	CCAGGCCTCG	CCCTCCAGCT	CAAGGCGGGA	CAGGTGCCCT	2280
	AGAGTAGCCT	GCATCCAGGG	ACACACCAGC	TGGGTACCAA	CATGTCCGGA	GCCACATGGA	2340
	CAGAGGCCGG	CTCGGCCAC	CCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	CCTCTGTCCC	2400
20	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA	CACCCTGCCC	CCATCCCCGG	ATGAGCTGAC	2460
	CAAGAACCAG	GTCAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCCAGCG	ACATCGCCGT	2520
	GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG	ACCACGCCTC	CCGTGCTGGA	2580
	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA	GCTCACCCTG	GACAAGAGCA	GGTGGCAGCA	2640
	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGCTCTG	CACAACCACT	ACACGCAGAA	2700
25	GAGCCTCTCC	CTGTCTCCGG	GTAATGAGT	GCGACGGCCG	GCAAGCCCC	GCTCCCCGGG	2760
	CTCTCGCGGT	CGCACGAGGA	TGCTTGGCAC	GTACCCCTG	TACATACTTC	CCGGGCGCCC	2820
	AGCATGGA	TAAAGCACC	AGCGCTGCCC	TGGGCCCCCTG	CGAGACTGTG	ATGGTTCTTT	2880
	CCACGGGTCA	GGCCGAGTCT	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	GGGTCCCACT	2940
	GTCCCCACAC	TGGCCCAGGC	TGTGCAGGTG	TGCCTGGGCC	CCTAGGGTG	GGGCTCAGCC	3000
30	AGGGGCTGCC	CTCGGCAGGG	TGGGGGCAATT	GCCAGCGTGG	CCCTCCCTCC	AGCAGCACCT	3060
	GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCTGGG	GACAGACACA	CAGCCCTGC	3120
	CTCTGTAGGA	GACTGTCTCTG	TTCTGTGAGC	GCCCTGTCC	TCCCGACCTC	CATGCCCACT	3180
	CGGGGGCATG	CCTAGTCCAT	GTGCGTAGGG	ACAGGCCCTC	CCTCACCCAT	CTACCCCCAC	3240
	GGCACTAACC	CCTGGCTGCC	CTGCCCAGCC	TCGCACCCGC	ATGGGGACAC	AACCGACTCC	3300
35	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GACTGGTGCA	GATGCCACA	CACACATCA	3360
	GCCCAGACCC	GTTCACAAA	CCCCGCACTG	AGGTTGGCCG	GCCACACGGC	CACCACACAC	3420
	ACACGTGCAC	GCCTCACACA	CGGAGCCTCA	CCCGGGCGAA	CTGCACAGCA	CCCAGACCAG	3480
	AGCAAGGTCC	TCGCACACGT	GAACACTCCT	CGGACACAGG	CCCCACAGAG	CCCCACGCGG	3540
	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	TCAGACAAAC	3600
40	CCAGCCCTCC	TCTCACAAGG	GTGCCCCCTG	AGCCGCCACA	CACACACAGG	GGATCACACA	3660
	CCACGTACG	TCCCTGGCCC	TGGCCCACTT	CCCAGTGCCG	CCCTTCCCTG	CAGGACGGAT	3720
	CAGCCTCGAC	TGTGCCTTCT	AGTTGCCAGC	CATCTGTTGT	TTGCCCCCTC	CCCGTGCCTT	3780
	CCTTGACCCT	GGAAGGTGCC	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	GAAATTGCAT	3840
	CGCATTGTCT	GAGTAGGTGT	CATTCTATTC	TGGGGGGTGG	GGTGGGGCAG	GACAGCAAGG	3900
45	GGGAGGATTG	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	ATGGCTTCTG	3960
	AGGCGGAAAG	AACCAGCTGG	GGCTCTAGGG	GGTATCCCCA	CGCGCCCTGT	AGCGGCGCAT	4020
	TAAGCGCGGC	GGGTGTGGTG	GTTACGCGCA	GCGTGACCGC	TACACTTGCC	AGCGCCCTAG	4080
	CGCCCGCTCC	TTTCGCTTTC	TTCCCTTCCT	TTCTCGCCAC	GTTGCGCGGG	CCTCTCAAAA	4140
	AAGGGAAAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	CTAACTCCGC	4200
50	CCATCCCGCC	CCTAACTCCG	CCCAGTTCGG	CCCATTCTCC	GCCCCATGGC	TGACTAATTT	4260
	TTTTTATTTA	TGCAGAGGCC	GAGGCCGCT	CGGCCTCTGA	GCTATTCCAG	AAGTAGTGAG	4320
	GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	GCTGCGATTT	4380
	CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	CCCGCTGCCA	4440
	TCATGGTTCC	ACCATGGAAC	TGCATCGTCG	CCGTGTCCCA	AAATATGGGG	ATTGGCAAGA	4500
55	ACGGAGACCT	ACCTTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	AGAATGACCA	4560
	CAACCTCTTC	AGTGGAAGGT	AAACAGAATC	TGGTGATTAT	GGGTAGGAAA	ACCTGGTTCT	4620
	CCATTCTCTGA	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	AGTAGAGAAC	4680
	TCAAAGAACC	ACCACGAGGA	GCTCATTTC	TTGCCAAAAG	TTGGGATGAT	GCCTTAAGAC	4740
	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	GGAGGCAGTT	4800

	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	ACAAGGATCA	4860
	TGCAGGAATT	TGAAAGTGAC	ACGTTTTTCC	CAGAAATTGA	TTTGGGGAAA	TATAAACTTC	4920
	TCCCAGAATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	AAGTATAAGT	4980
	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	GCTCCCCTCC	5040
5	TAAAGCTATG	CATTTTTATA	AGACCATGGG	ACTTTTGCTG	GCTTTAGATC	TCTTTGTGAA	5100
	GGAACCTTAC	TTCTGTGGTG	TGACATAATT	GGACAACTA	CCTACAGAGA	TTTAAAGCTC	5160
	TAAGGTAAAT	ATAAAATTTT	TAAGTGTATA	ATGTGTTAAA	CTACTGATTC	TAATTGTTTTG	5220
	TGTATTTTAG	ATTCCAACCT	ATGGAACCTG	TGAATGGGAG	CAGTGGTGGA	ATGCCTTTAA	5280
	TGAGGAAAAC	CTGTTTTGCT	CAGAAGAAAT	GCCATCTAGT	GATGATGAGG	CTACTGCTGA	5340
10	CTCTCAACAT	TCTACTCCTC	CAAAAAAGAA	GAGAAAAGTA	GAAGACCCCA	AGGACTTTCC	5400
	TTCAGAATTG	CTAAGTTTTT	TGAGTCATGC	TGTGTTTTAGT	AATAGAACTC	TTGCTTGCTT	5460
	TGCTATTTAC	ACCACAAAGG	AAAAAGCTGC	ACTGCTATAC	AAGAAAATTA	TGGAAAAATA	5520
	TTCTGTAACC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	TTTTCTTTAC	5580
	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACTATGCT	CAAAAATTGT	GTACCTTTAG	5640
15	CTTTTAAATT	TGTAAAGGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCC	TGACTAGAGA	5700
	TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTTACTTGC	TTTAAAAAAC	CTCCACACCC	5760
	TCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	TTTATTGCAG	5820
	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	GCATTTTTTT	5880
	CACTGCATTG	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	GTCTGGATCG	5940
20	GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCCAC	CCCAACTTGT	6000
	TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTT	ACAAATAAAG	6060
	CATTTTTTTC	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	TCTTATCATG	6120
	TCTGTATACC	GTGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTTCCTG	6180
	TGTGAAATTG	TTATCCGCTC	ACAATTCCAC	ACAACATACG	AGCCGGAAGC	ATAAAGTGTA	6240
25	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAA	TGCGTTGCGC	TCACTGCCCCG	6300
	CTTTCCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA	6360
	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCCG	CTTCTCGCT	CACTGACTCG	CTGCGCTCGG	6420
	TCGTTCCGGT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	6480
	AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAAG	CCAGCAAAAAG	GCCAGGAACC	6540
30	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	6600
	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGCGCT	6660
	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	6720
	TGTCCGCCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTCTCA	ATGCTCACGC	TGTAGGTATC	6780
	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	6840
35	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGTA	AGACACGACT	6900
	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	6960
	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTTGGTA	7020
	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	7080
	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	7140
40	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAACG	7200
	AAAACTCACG	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	ACCTAGATCC	7260
	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG	7320
	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTTCAT	7380
	CCATAGTTGC	CTGACTCCCC	GTCGTGTAGA	TAACCTACGAT	ACGGGAGGGC	TTACCATCTG	7440
45	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	TTATCAGCAA	7500
	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	TCCGCCTCCA	7560
	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCCGCCAGT	AATAGTTTGC	7620
	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTCACG	CTCGTCGTTT	GGTATGGCTT	7680
	CATTACAGTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTGTGCAAAA	7740
50	AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTGAGAAG	TAAGTTGGCC	GCAGTGTTAT	7800
	CACTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	GTAAGATGCT	7860
	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGCGACCGA	7920
	GTTGCTCTTG	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	ACTTTAAAAG	7980
	TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC	GAAAACCTC	AAGGATCTTA	CCGCTGTGGA	8040
55	GATCCAGTTC	GATGTAACCC	ACTCGTGAC	CCAACCTGATC	TTCAGCATCT	TTTACTTTCA	8100
	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAAG	GGAATAAGGG	8160
	CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTTCA	ATATTATTGA	AGCATTATATC	8220
	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG	8280
	GGGTTCCGCG	CACATTTCCC	CGAAAAGTGC	CACCTGACGT	CCBRAAG		8327

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 8897 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

	GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGCAC CATGAAGTTG CCTGTTAGGC	60
15	TGTTGGTGCT GATGTTCTGG ATTCCTGCTT CCAGCAGTGA TGTTTGTGATG ACCCAAATTC	120
	CAGTCTCCCT GCCTGTCAGT CTTGGAGATC AAGCGTCCAT CTCTTGACAG TCTAGTCAGA	180
	TCATTGTACA TAATAATGGC AACACCTATT TAGAATGGTA CCTGCAGAAA CCAGGCCAGT	240
	CTCCACAGCT CCTGATCTAC AAAGTTTCCA ACCGATTTTC TGGGGTCCCA GACAGGTTCA	300
	GCGGCAGTGG ATCAGGGACA GATTTCACAC TCAAGATCAG CAGAGTGGAG GCTGAGGATC	360
20	TGGGAGTTTA TTACTGCTTT CAAGGTTTCC ATGTTCCATT CACGTTCCGC TCGGGGACAA	420
	AGTTGGAAAT AAAACGTAAG TCTCGAGTCT CTAGATAACC GGTCAATCGA TTGGAATTCT	480
	AAACTCTGAG GGGGTCGGAT GACGTGGCCA TTCTTTGCCT AAAGCATTGA GTTTACTGCA	540
	AGGTCAGAAA AGCATGCAAA GCCCTCAGAA TGGCTGCAAA GAGCTCCAAC AAAACAATTT	600
	AGAAGTTTAT TAAGGAATAG GGGGAAGCTA GGAAGAACT CAAAACATCA AGATTTTAAA	660
25	TACGCTTCTT GGTCTCCTTG CTATAATTAT CTGGGATAAG CATGCTGTTT TCTGTCTGTC	720
	CCTAACATGC CCTTATCCGC AAACAACACA CCCAAGGGCA GAACTTTGTT ACTTAAACAC	780
	CATCCTGTTT GCTTCTTTCC TCAGGAACCTG TGGCTGCACC ATCTGTCTTC ATCTTCCGC	840
	CATCTGATGA GCAGTTGAAA TCTGGAACCTG CCTCTGTTGT GTGCCTGCTG AATAACTTCT	900
	ATCCCAGAGA GGCCAAAGTA CAGTGGGAAG TGGATAACGC CCTCCAATCG GGTAACCTCC	960
30	AGGAGAGTGT CACAGAGCAG GAGAGCAAGG ACAGCACCTA CAGCCTCAGC AGCACCCTGA	1020
	CGCTGAGCAA AGCAGACTAC GAGAAACACA AAGTCTACGC CTGCGAAGTC ACCCATCAGG	1080
	GCCTGAGCTC GCCCGTCACA AAGAGCTTCA ACAGGGGAGA GTGTTAGAGG GAGAAGTGCC	1140
	CCCACCTGCT CCTCAGTTCC AGCCTGACCC CCTCCCATCC TTTGGCCTCT GACCCTTTT	1200
	CCACAGGGGA CCTACCCCTA TTGCGGTCTT CCAGCTCATC TTTACCTCA CCCCCCTCCT	1260
35	CCTCCTTGGC TTTAATTATG CTAATGTTGG AGGAGAATGA ATAAATAAAG TGAATCTTTG	1320
	CACCTGTGGT TTCTCTCTTT CCTCATTTAA TAATTATTAT CTGTTGTTTT ACCAACTACT	1380
	CAATTTCTCT TATAAGGGAC TAAATATGTA GTCATCTTAA GGCACGTAAC CATTTATAAA	1440
	AATCATCCCT CATTTCTATT TACCCATCA TCCTCTGCAA GACAGTCCTC CCTCAAACCC	1500
	ACAAGCCTTC TGTCCTCACA GTCCCTGCGG CCATGGTAGG AGAGACTTGC TTCCTTGTTT	1560
40	TCCCCTCTC AGCAAGCCCT CATAGTCCTT TTTAAGGGTG ACAGGTCTTA CAGTCATATA	1620
	TCCTTTGATT CAATTCCTTG AGAATCAACC AAAGCAAATT TTTCAAAAGA AGAAACCTGC	1680
	TATAAAGAGA ATCATTCAAT GCAACATGAT ATAAATAAAC AACACAATAA AAGCAATTAA	1740
	ATAAACAAAC AATAGGGAAA TGTTTAAGTT CATCATGGTA CTTAGACTTA ATGGAATGTC	1800
	ATGCCTTATT TACATTTTAA AACAGGTAAT GAGGGACTCC TGTCTGCCAA GGGCCGTATT	1860
45	GAGTACTTTC CACAACCTAA TTTAATCCAC ACTATACTGT GAGATTAAAA ACATTCAATTA	1920
	AAATGTTGCA AAGGTTCTAT AAAGCTGAGA GACAAATATA TTCTATAACT CAGCAATCCC	1980
	ACTTCTAGAT GACTGAGTGT CCCCACCCAC CAAAAAATA TGCAAGAATG TTCAAAGCAG	2040
	CTTTATTTAC AAAAGCCAAA AATTGGAAAT AGCCCGATTG TCCAACAATA GAATGAGTTA	2100
	TTAAACTGTG GTATGTTTAT ACATTAGAAT ACCCAATGAG GAGAATTAAC AAGCTACAAC	2160
50	TATACCTACT CACACAGATG AATCTCATAA AAATAATGTT ACATAAGAGA AACTCAATGC	2220
	AAAAGATATG TTCTGTATGT TTTCAATCCAT ATAAAGTTCA AAACCAGGTA AAAATAAAGT	2280
	TAGAAATTTG GATGGAAATT ACTCTTAGCT GGGGGTGGGC GAGTTAGTGC CTGGGAGAAG	2340
	ACAAGAAGGG GCTTCTGGGG TCTTGGTAAT GTTCTGTTCC TCGTGTGGGG TTGTGCAGTT	2400
	ATGATCTGTG CACTGTTCTG TATACACATT ATGCTTCAAA ATAACCTCAC ATAAAGAACA	2460
55	TCTTATACCC AGTTAATAGA TAGAAGAGGA ATAAGTAATA GGTCAAGACC AACGCAGCTG	2520
	GTAAGTGGGG GCCTGGGATC AAATAGCTAC CTGCCTAATC CTGCCCWCTT GAGCCCTGAA	2580
	TGAGTCTGCC TTCCAGGGCT CAAGGTGCTC AACAAAACAA CAGGCCTGCT ATTTTCCTGG	2640
	CATCTGTGCC CTGTTTGGCT AGCTAGGAGC ACACATACAT AGAAATTAAA TGAACACAGC	2700
	CTTCAGCAAG GGGACAGAGG ACAGAATTAA CCTTGCCCAG AACTGGGAAA CCCATGTATG	2760

	AACACTCACA	TGTTTGGGAA	GGGGGAAGGG	CACATGTAAA	TGAGGACTCT	TCCTCATTCT	2820
	ATGGGGCACT	CTGGCCCTGC	CCCTCTCAGC	TACTCATCCA	TCCAACACAC	CTTTCTAAGT	2880
	ACCTCTCTCT	GCCTACACTC	TGAAGGGGTT	CAGGAGTAAC	TAACACAGCA	TCCCTTCCCT	2940
	CAAATGACTG	ACAATCCCTT	TGTCCTGCTT	TGTTTTTCTT	TCCAGTCAGT	ACTGGGAAAAG	3000
5	TGGGGAAGGA	CAGTCATGGA	GAAACTACAT	AAGGAAGCAC	CTTGCCCTTC	TGCCTCTTGA	3060
	GAATGTTGAT	GAGTATCAAA	TCTTTCAAAC	TTTGGAGGTT	TGAGTAGGGG	TGAGACTCAG	3120
	TAATGTCCCT	TCCAATGACA	TGAACCTGCT	CACTCATCCC	TGGGGGCCAA	ATTGAACAAAT	3180
	CAAAGGCAGG	CATAATCCAG	TTATGAATTC	TTGCGGCCGC	TTGCTAGCTT	CACGTGTTGG	3240
	ATCCAACCGC	GGAAGGGCCC	TATTCTATAG	TGTCACCTAA	ATGCTAGAGC	TCGCTGATCA	3300
10	GCCTCGACTG	TGCCTTCTAG	TTGCCAGCCA	TCTGTGTGTT	GCCCCCTCCC	CGTGCCTTCC	3360
	TTGACCCTTG	AAGTGCCAC	TCCCCTGTC	TCTTCTAAT	AAAATGAGGA	AATTGCATCG	3420
	CATTGTCTGA	GTAGGTGTCA	TTCTATTCTG	GGGGGTGGGG	TGGGGCAGGA	CAGCAAGGGG	3480
	GAGGATTGGG	AAGACAATAG	CAGGCATGCT	GGGGATGCGG	TGGGCTCTAT	GGCTTCTGAG	3540
	GCGGAAAGAA	CCAGCTGGGG	CTCTAGGGGG	TATCCCCACG	CGCCCTGTAG	CGGCGCATT	3600
15	AGCGCGCGCG	GTGTGGTGGT	TACGCGCAGC	GTGACCGCTA	CACTTGCCAG	CGCCCTAGCG	3660
	CCCGCTCCTT	TCGCTTTCTT	CCCTTCTTTT	CTCGCCACGT	TCGCCGGGCC	TCTCAAAAAA	3720
	GGGAAAAAAA	GCATGCATCT	CAATTAGTCA	GCAACCATAG	TCCCCGCCCT	AACTCCGCC	3780
	ATCCCGCCCC	TAACTCGCC	CAGTTCCGCC	CATTCTCCGC	CCCATGGCTG	ACTAATTTTT	3840
	TTTATTTATG	CAGAGGCCGA	GGCCGCTCG	GCCTCTGAGC	TATTCCAGAA	GTAGTGAGGA	3900
20	GGCTTTTTTG	GAGGCCTAGG	CTTTTGCAAA	AAGCTTGGAC	AGCTCAGGGC	TGCGATTTCG	3960
	CGCCAAACTT	GACGGCAATC	CTAGCGTGAA	GGCTGGTAGG	ATTTTATCCC	CGCTGCCATC	4020
	ATGGTTTCGAC	CATTGAACTG	CATCGTCGCC	GTGTCCCAAA	ATATGGGGAT	TGGCAAGAAC	4080
	GGAGACCTAC	CCTGGCCTCC	GCTCAGGAAC	GAGTTCAAGT	ACTTCCAAAG	AATGACCACA	4140
25	ACCTCTTCAG	TGGAAGGTAA	ACAGAATCTG	GTGATTATGG	GTAGGAAAAC	CTGGTTCTCC	4200
	ATTCTTGAGA	AGAATCGACC	TTTAAAGGAC	AGAATTAATA	TAGTTCTCAG	TAGAGAACTC	4260
	AAAGAACCAC	CACGAGGAGC	TCATTTTCTT	GCCAAAAGTT	TGGATGATGC	CTTAAGACTT	4320
	ATTGAACAAC	CGGAATTGGC	AAGTAAAGTA	GACATGGTTT	GGATAGTCGG	AGGCAGTTCT	4380
	GTTTACCAGG	AAGCCATGAA	TCAACCAGGC	CACCTTAGAC	TCTTTGTGAC	AAGGATCATG	4440
	CAGGAATTTG	AAAGTGACAC	GTTTTTCCCA	GAAATTGATT	TGGGGAAATA	TAAACTTCTC	4500
30	CCAGAATACC	CAGGCGTCTT	CTCTGAGGTC	CAGGAGGAAA	AAGGCATCAA	GTATAAGTTT	4560
	GAAGTCTACG	AGAAGAAAGA	CTAACAGGAA	GATGCTTTCA	AGTTCTCTGC	TCCCCTCCTA	4620
	AAGCTATGCA	TTTTTATAAG	ACCATGGGAC	TTTTGCTGGC	TTTAGATCTC	TTTGTGAAGG	4680
	AACCTTACTT	CTGTGGTGTG	ACATAATTGG	ACAAACTACC	TACAGAGATT	TAAAGCTCTA	4740
	AGGTAAATAT	AAAATTTTTA	AGTGATATAAT	GTGTTAAACT	ACTGATTCTA	ATTGTTTGTG	4800
35	TATTTTAGAT	TCCAACCTAT	GGAAGTATG	AATGGGAGCA	GTGGTGGAAT	GCCTTTAATG	4860
	AGGAAAACCT	GTTTTTGCTCA	GAAGAAATGC	CATCTAGTGA	TGATGAGGCT	ACTGCTGACT	4920
	CTCAACATTC	TACTCCTCCA	AAAAAGAAGA	GAAAGGTAGA	AGACCCCAAG	GACTTTCCTT	4980
	CAGAATTGCT	AAGTTTTTTG	AGTCATGCTG	TGTTTAGTAA	TAGAACTCTT	GCTTGCTTTG	5040
	CTATTTACAC	CACAAAGGAA	AAAGCTGCAC	TGCTATACAA	GAAAATTATG	GAAAAATATT	5100
40	CTGTAACCTT	TATAAGTAGG	CATAACAGTT	ATAATCATAA	CATACTGTTT	TTTCTTACTC	5160
	CACACAGGCA	TAGAGTGTCT	GCTATTAATA	ACTATGCTCA	AAAATTGTGT	ACCTTTAGCT	5220
	TTTTAATTTG	TAAAGGGGTT	AATAAGGAAT	ATTTGATGTA	TAGTGCCTTG	ACTAGAGATC	5280
	ATAATCAGCC	ATACCACATT	TGTAGAGGTT	TTACTTGCTT	TAAAAAACCT	CCCACACCTC	5340
	CCCCTGAACC	TGAAACATAA	AATGAATGCA	ATTGTTGTTG	TTAACTTGTT	TATTGCAGCT	5400
45	TATAATGGTT	ACAAATAAAG	CAATAGCATC	ACAAATTTCA	CAAATAAAGC	ATTTTTTTTCA	5460
	CTGCATTCTA	GTTGTGGTTT	GTCCAAACTC	ATCAATGTAT	CTTATCATGT	CTGGATCGGC	5520
	TGGATGATCC	TCCAGCGCGG	GGATCTCATG	CTGGAGTTCT	TCGCCCACCC	CAACTTGTTT	5580
	ATTGCAGCTT	ATAATGGTTA	CAAATAAAGC	AATAGCATCA	CAAATTTTAC	AAATAAAGCA	5640
	TTTTTTTTTAC	TGCATTCTAG	TTGTGGTTTG	TCCAAACTCA	TCAATGTATC	TTATCATGTC	5700
50	TGTATACCGT	CGACCTCTAG	CTAGAGCTTG	GCGTAATCAT	GGTCATAGCT	GTTTCCTGTG	5760
	TGAAATTTGT	ATCCGCTCAC	AATTCCACAC	AACATACGAG	CCGGAAGCAT	AAAGTGTAAG	5820
	GCCTGGGGTG	CCTAATGAGT	GAGCTAACTC	ACATTAATTG	CGTTGCGCTC	ACTGCCCGCT	5880
	TTCCAGTCGG	GAAACCTGTC	GTGCCAGCTG	CATTAATGAA	TCGGCCAACG	CGCGGGGAGA	5940
	GGCGGTTTGC	GTATTGGGCG	CTCTTCCGCT	TCCTCGCTCA	CTGACTCGCT	GCGCTCGGTC	6000
55	GTTCCGCTGC	GGCGAGCGGT	ATCAGCTCAC	TCAAAGGCGG	TAATACGGTT	ATCCACAGAA	6060
	TCAGGGGATA	ACGCAGGAAA	GAACATGTGA	GCAAAAGGCC	AGCAAAAGGC	CAGGAACCGT	6120
	AAAAAGGCCG	CGTTGCTGGC	GTTTTTCCAT	AGGCTCCGCC	CCCCTGACGA	GCATCACAAA	6180
	AATCGACGCT	CAAGTCAGAG	GTGGCGAAAC	CCGACAGGAC	TATAAAGATA	CCAGGCGTTT	6240
	CCCCCTGGAA	GCTCCCTCGT	GCGCTCTCCT	GTTCCGACCC	TGCCGCTTAC	CGGATACCTG	6300

TCCGCTTTC TCCCTTCGGG AAGCGTGGCG CTTTCTCAAT GCTCACGCTG TAGGTATCTC 6360
 AGTTCGGTGT AGGTCGTTCC CTCCAAGCTG GGCTGTGTGC ACGAACCCCC CGTTCAGCCC 6420
 GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG ACACGACTTA 6480
 TCGCCACTGG CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT AGGCGGTGCT 6540
 5 ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC 6600
 TGCGCTCTGC TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA 6660
 CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC GCGCAGAAAA 6720
 AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA GTGGAACGAA 6780
 AACTCACGTT AAGGGATTTT GGTCAATGAG TATCAAAAA GGATCTTCAC CTAGATCCTT 6840
 10 TTAATAATAA AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC 6900
 AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT TCGTTCATCC 6960
 ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT ACCATCTGGC 7020
 CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATTT ATCAGCAATA 7080
 AACCAGCCAG CCGGAAGGGC CGAGCGCAGA AGTGGTCTCG CAACTTTATC CGCCTCCATC 7140
 15 CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC 7200
 AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTACGCT CGTCGTTTGG TATGGCTTCA 7260
 TTCAGTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT GTGCAAAAAA 7320
 GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC AGTGTATCA 7380
 CTCATGGTTA TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT AAGATGCTTT 7440
 20 TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT 7500
 TGCTCTTGCC CGGCGTCAAT ACGGGATAAT ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG 7560
 CTCATCATTTG GAAAAAGTTT TFCGGGGCGA AAACCTCTCA GGATCTTACC GCTGTTGAGA 7620
 TCCAGTTTGA TGTAACCCAC TCGTGACCCC AACTGATCTT CAGCATCTTT TACTTTTACC 7680
 AGCGTTTCTG GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG AATAAGGGCG 7740
 25 ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTCATAT ATTATTTGAAG CATTATATCAG 7800
 GGTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG 7860
 GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCG ACGGATCGGG AGATCTGCTA 7920
 GCCCGGGTGA CCTGAGGCGC GCCGCTTCG AATAGCCAGA GTAACCTTTT TTTTAAATT 7980
 TATTTTATTT TATTTTGTAG ATGGAGTTTG GCGCCGATCT CCGGATCCCC TATGGTCGAC 8040
 30 TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATCTGCTCC CTGCTTGTGT 8100
 GTTGGAGGTC GCTGAGTAGT GCGCGAGCAA AATTAAAGCT ACAACAAGGC AAGGCTTGAC 8160
 CGACAATTGC ATGAAGAATC TGCTTAGGGT TAGGCGTTTT GCGCTGCTTC GCGATGTACG 8220
 GGCCAGATAT ACGCGTTGAC ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG 8280
 GTCATTAGTT CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACCTACGG TAAATGGCCC 8340
 35 GCCTGGCTGA CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT 8400
 AGTAACGCCA ATAGGGACTT TCCATTGACG TCAATGGGTG GACTATTTAC GGTAAACTGC 8460
 CCACTTGGCA GTACATCAAG TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA 8520
 CGGTAAATGG CCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG 8580
 GCAGTACATC TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT 8640
 40 CAATGGGCGT GGATAGCGGT TTGACTCAGC GGGATTTCCA AGTCTCCACC CCATTGACGT 8700
 CAATGGGAGT TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAATGTCT GTAACAACCTC 8760
 CGCCCCATTG ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC 8820
 TCTCTGGCTA ACTAGAGAAC CCACTGCTTA CTGGCTTATC GAAATTAATA CGACTCACTA 8880
 TAGGGAGACC CAAGCTT 8897

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8321 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC GGTCAATCGA 60
 TTGGAATTCT TCGGCGCGCT TGCTAGCCAC CATGGAGTTG TGGTTAAGCT TGGTCTTCCT 120

	TGTCCTTGTT	TTAAAAGGTG	TCCAGTGTGA	AGTGCAACTG	GTGGAGTCTG	GGGGAGGCTT	180
	AGTGCAGCCT	GGAGGGTCCC	TGCGACTTTC	CTGTGCTGCA	TCTGGATTCC	CGTTCAGTGA	240
	CTATTACATG	TATTGGGTTC	GCCAGGCTCC	AGGCAAGGGA	CTGGAGTGGG	TCTCATACAT	300
	TAGTCAAGAT	GGTGATATAA	CCGACTATGC	AGACTCCGTA	AAGGGTCGAT	TCACCATCTC	360
5	CAGAGCAAT	GCAAAGAACA	GCCTGTACCT	GCAAATGAAC	AGCCTGAGGG	ACGAGGACAC	420
	AGCCGTGTAT	TACTGTGCAA	GAGGCCTGGC	GGACGGGGCC	TGGTTTGCTT	ACTGGGGCCA	480
	AGGGACTCTG	GTCACGGTCT	CTTCCGCTAG	CACCAAGGGC	CCATCGGTCT	TCCCCCTGGC	540
	ACCCTCCTCC	AAGAGCACCT	CTGGGGGCAC	AGCGGCCCTG	GGCTGCCTGG	TCAAGGACTA	600
	CTTCCCCGAA	CCGGTGACGG	TGTCGTGGAA	CTCAGGCGCC	CTGACCAGCG	GCGTGCACAC	660
10	CTTCCCGGCT	GTCCTACAGT	CCTCAGGACT	CTACTCCCTC	AGCAGCGTGG	TCACCGTGCC	720
	CTCCAGCAGC	TTGGGCACCC	AGACCTACAT	CTGCAACGTG	AATCACAAGC	CCAGCAACAC	780
	CAAGGTGGAG	AAGAAAGTTG	GTGAGAGGCC	AGCAAGGGA	GGGAGGGTGT	CTGCTGGAAG	840
	CCAGGCTCAG	CGCTCCTGCC	TGGACGCATC	CCGGCTATGC	AGCCCCAGTC	CAGGGCAGCA	900
	AGGCAGGCCC	CGTCTGCCTC	TTCACCCGGA	GGCCTCTGCC	CGCCCCACTC	ATGCTCAGGG	960
15	AGAGGGTCTT	CTGGCTTTTT	CCCAGGCTC	TGGGCAGGCA	CAGGCTAGGT	GCCCCCTAAC	1020
	CAGGCCCTGC	ACACAAAGGG	GCAGGTGCTG	GGCTCAGACC	TGCCAAGAGC	CATATCCGGG	1080
	AGGACCCTGC	CCCTGACCTA	AGCCCACCCC	AAAGGCCAAA	CTCTCCACTC	CCTCAGCTCG	1140
	GACACCTTCT	CTCCTCCCAG	ATTCCAGTAA	CTCCCAATCT	TCTCTCTGCA	GAGCCCAAAT	1200
	CTTGTGACAA	AACCTCACACA	TGCCACCCGT	GCCCAGGTAA	GCCAGCCCAG	GCCTCGCCCT	1260
20	CCAGCTCAAG	GCGGGACAGG	TGCCCTAGAG	TAGCCTGCAT	CCAGGGACAC	ACCACGTGGG	1320
	TACCAACATG	TCCGGAGCCA	CATGGACAGA	GGCCGGCTCG	GCCCACCCTC	TGCCCTGAGA	1380
	GTGACCGCTG	TACCAACCTC	TGTCCTTACA	GGGCAGCCCC	GAGAACCACA	GGTGACACC	1440
	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG	AACCAGGTCA	GCCTGACCTG	CCTGGTCAAA	1500
	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	1560
25	TACAAGACCA	CGCCTCCCGT	GCTGGACTCC	GACGGTCTCT	TCTTCTCTTA	CAGCAAGCTC	1620
	ACCGTGGACA	AGAGCAGGTG	GCAGCAGGGG	AACGTCTTCT	CATGCTCCGT	GATGCATGAG	1680
	GCTCTGCACA	ACCACTACAC	GCAGAAGAGC	CTCTCCCTGT	CTCCGGGTAA	ATGAGTGCGA	1740
	CGGCCGGCAA	GCCCCCGCTC	CCCGGGCTCT	CGCGGTGCGA	CGAGGATGCT	TGGCACGTAC	1800
	CCCCTGTACA	TACTTCCCGG	GCGCCCAGCA	TGGAAATAAA	GCACCCAGCG	CTGCCCTGGG	1860
30	CCCCTGCGAG	ACTGTGATGG	TTCTTTCCAC	GGGTCCAGCC	GAGTCTGAGG	CCTGAGTGGC	1920
	ATGAGGGAGG	CAGAGCGGGT	CCCACGTGCC	CCACACTGGC	CCAGGCTGTG	CAGGTGTGCC	1980
	TGGGCCCCCT	AGGGTGGGGC	TCAGCCAGGG	GCTGCCCTCG	GCAGGGTGGG	GGATTTGCCA	2040
	GCGTGGCCCT	CCCTCCAGCA	GCACCTGCCC	TGGGCTGGGC	CACGGGAAGC	CCTAGGAGCC	2100
	CCTGGGGACA	GACACACAGC	CCCTGCCTCT	GTAGGAGACT	GTCCGTGTTCT	GTGAGCGCCC	2160
35	CTGTCTCTCC	GACCTCCATG	CCCACCTCGG	GGCATGCCTA	GTCCATGTGC	GTAGGGACAG	2220
	GCCCTCCCTC	ACCCATCTAC	CCCCACGGCA	CTAACCCCTG	GCTGCCCTGC	CCAGCCTCGC	2280
	ACCCGCATGG	GGACACAACC	GACTCCGGGG	ACATGCACCT	TCGGGCCCTG	TGGAGGGACT	2340
	GGTGACAGATG	CCCACACACA	CACCTCAGCC	AGACCCTGTC	AACAAACCCC	GCACTGAGGT	2400
	TGGCCGGCCA	CACGGCCACC	ACACACACAC	GTGCACGCCT	CACACACGGA	GCCTCACCCG	2460
40	GGCGAACTGC	ACAGCACCCA	GACCAGAGCA	AGGTCTCTCG	ACACGTGAAC	ACTCCTCGGA	2520
	CACAGGCCCC	CACGAGCCCC	ACGCGGCACC	TCAAGGCCCA	CGAGCCTCTC	GGCAGCTTCT	2580
	CCACATGCTG	ACCTGCTCAG	ACAAACCCAG	CCCTCCTCTC	ACAAGGGTGC	CCCTGCAGCC	2640
	GCCACACACA	CACAGGGGAT	CACACACCAC	GTCACGTCCC	TGGCCCTGGC	CCACTTCCCA	2700
	GTGCCGCCCT	TCCCTGCAGG	ACGGATCAGC	CTCGACTGTG	CCTTCTAGTT	GCCAGCCATC	2760
45	TGTTGTTTGC	CCCTCCCCCG	TGCCCTTCTT	GACCCCTGGA	GGTGCCACTC	CCACTGTCTT	2820
	TTCTTAATAA	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCATT	CTATTCTGGG	2880
	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA	GACAATAGCA	GGCATGCTGG	2940
	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAACC	AGCTGGGGCT	CTAGGGGGTA	3000
	TCCCCACGCG	CCCTGTAGCG	GCGCATTAA	CGCGCGGGT	GTGGTGGTTA	CGCGCAGCGT	3060
50	GACCGCTACA	CTTGCCAGCG	CCCTAGCGCC	CGTCTCTTTC	GCTTTCTTCC	CTTCTTTTCT	3120
	CGCCACGTTT	GCCGGGCCCT	TCAAAAAAGG	GAAAAAAGC	ATGCATCTCA	ATTAGTCAGC	3180
	AACCATAGTC	CCGCCCTTAA	CTCCGCCCAT	CCCCCCCCTA	ACTCCGCCCA	GTTCCGCCCA	3240
	TTCTCCGCCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA	GAGGCCGAGG	CCGCCTCGGC	3300
	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG	CTTTTTTGGA	GGCCTAGGCT	TTTGCAAAAA	3360
55	GCTTGGACAG	CTCAGGGCTG	CGATTTCGCG	CCAAACTTGA	CGGCAATCCT	AGCGTGAAGG	3420
	CTGGTAGGAT	TTTATCCCCG	CTGCCATCAT	GGTTCGACCA	TTGAACTGCA	TCGTCGCCGT	3480
	GTCCCAAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC	TCAGGAACGA	3540
	GTTCAAGTAC	TTCCAAAGAA	TGACCACAAC	CTCTTCAGTG	GAAGGTAAAC	AGAATCTGGT	3600
	GATTATGGGT	AGGAAAACCT	GGTTCTCCAT	TCCTGAGAAG	AATCGACCTT	TAAAGGACAG	3660

	AATTAATATA	GTTCTCAGTA	GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	ATTTTCTTGC	3720
	CAAAAGTTTG	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	GTAAAGTAGA	3780
	CATGGTTTGG	ATAGTCGGAG	GCAGTTCGT	TTACCAGGAA	GCCATGAATC	AACCAGGCCA	3840
	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAATTTGAA	AGTGACACGT	TTTTCCAGAA	3900
5	AATTGATTTG	GGGAAATATA	AACCTTCTCC	AGAATACCCA	GGCGTCTCT	CTGAGGTCCA	3960
	GGAGGAAAAA	GGCATCAAGT	ATAAGTTTGA	AGTCTACGAG	AAGAAAGACT	AACAGGAAGA	4020
	TGCTTTCAAG	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	CATGGGACTT	4080
	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC	ATAATTGGAC	4140
	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAAATATAA	AATTTTTAAG	TGTATAATGT	4200
10	GTTAAACTAC	TGATTCTAAT	TGTTTGTGTA	TTTTAGATT	CAACCTATGG	AACTGATGAA	4260
	TGGGAGCAGT	GGTGGAATGC	CTTTAATGAG	GAACACCTGT	TTTGCTCAGA	AGAAATGCCA	4320
	TCTAGTGATG	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	AAAGAAGAGA	4380
	AAGGTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCTAA	GTTTTTTGAG	TCATGCTGTG	4440
	TTTAGTAATA	GAACCTTTC	TTGCTTTGCT	ATTTACACCA	CAAAGGAAAA	AGCTGCACTG	4500
15	CTATACAAGA	AAATTATGGA	AAAATATTCT	GTAACCTTTA	TAAGTAGGCA	TAACAGTTAT	4560
	AATCATAACA	TACTGTTTTT	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	TATTAATAAC	4620
	TATGCTCAAA	AATTGTGTAC	CTTTAGCTTT	TTAATTTGTA	AAGGGGTTAA	TAAGGAATAT	4680
	TTGATGTATA	GTGCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTTG	TAGAGGTTTT	4740
	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA	TGAATGCAAT	4800
20	TGTTGTGTGT	AACCTGTTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAAGCA	ATAGCATCAC	4860
	AAATTTTACA	AATAAAGCAT	TTTTTTTCACT	GCATTCTAGT	TGTGGTTTTGT	CCAAACTCAT	4920
	CAATGTATCT	TATCATGTCT	GGATCGGCTG	TATGATCCTC	CAGCGCGGGG	ATCTCATGCT	4980
	GGAGTTCCTC	GCCCCACCCA	ACTTGTTTTAT	GATCAGCTTAT	AATGGTTACA	AATAAAGCAA	5040
	TAGCATCACA	AATTTTCAAA	ATAAAGCATT	TTTTTCACTG	CATTCTAGTT	GTGGTTTGTC	5100
25	CAAACTCATC	AATGTATCTT	ATCATGTCTG	TATACCGTCG	ACCTCTAGCT	AGAGCTTGGC	5160
	GTAATCATGG	TCATAGCTGT	TTCCTGTGTG	AAATTGTTAT	CCGCTCACAA	TTCCACACAA	5220
	CATACGAGCC	GGAAGCATAA	AGTGTAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAACTCAC	5280
	ATTAATTGCG	TTGCGCTCAC	TGCCCGCTTT	CCAGTCGGGA	AACCTGTCTG	GCCAGCTGCA	5340
30	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCCT	ATTGGGCGCT	CTTCCGCTTC	5400
	CTCGCTCACT	GACTCGCTGC	GCTCCGTCGT	TCCGCTGCGG	CGAGCGGTAT	CAGCTCACTC	5460
	AAAGGCGGTA	ATACGCTTAT	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	5520
	AAAAGGCCAG	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	TTTTCCATAG	5580
	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT	GGCGAAACCC	5640
	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC	TCCCTCGTGC	GCTCTCCTGT	5700
35	TCCGACCCTG	CCGCTTACCG	GATACCTGTG	CGCCTTTCTC	CCTTCGGGAA	GCGTGGCGCT	5760
	TTCTCAATGC	TCACGCTGTA	GGTATCTCAG	TTCCGGTGTAG	GTCGTTGCGT	CCAAGCTGGG	5820
	CTGTGTGCAC	GAACCCCCCG	TTCAGCCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT	5880
	TGAGTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG	GTAACAGGAT	5940
	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC	CTAACTACGG	6000
40	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	6060
	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAACA	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	6120
	TTGCAAGCAG	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	6180
	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	TCATGAGATT	6240
	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATTAATAA	TGAAGTTTTA	AATCAATCTA	6300
45	AAGTATATAT	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	6360
	CTCAGCGATC	TGTCTATTTT	GTTTCATCCAT	AGTTGCCTGA	CTCCCCGTCG	TGTAGATAAC	6420
	TACGATACCG	GAGGGCTTAC	CATCTGGCCC	CAGTGTGCTG	ATGATACCGC	GAGACCCACG	6480
	CTCACCGGCT	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGAAAGGGCCG	AGCGCAGAAG	6540
	TGGTCCTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	AAGCTAGAGT	6600
50	AAGTAGTTTC	CCAGTTAATA	GTTTGCAGCA	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT	6660
	GTCACGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	6720
	TACATGATCC	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC	TTCCGGTCTC	CGATCGTTGT	6780
	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC	ATAATTCTCT	6840
	TACTGTCAAT	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	6900
55	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG	CTCTTGCCCG	GCGTCAATAC	GGGATAATAC	6960
	CGCGCCACAT	AGCAGAACTT	TAAAAGTGCT	CATCATTGGA	AAACGTCTCT	CGGGGCGAAA	7020
	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTTCGATG	TAACCCACTC	GTGCACCCAA	7080
	CTGATCTTCA	GCATCTTTTA	CTTTCACCAG	CGTTTCTGGG	TGAGCAAAAA	CAGGAAGGCA	7140
	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAATGT	TGAATACTCA	TACTCTTCTT	7200

	TTTTCAATAT	TATTGAAGCA	TTTATCAGGG	TTATTGTCTC	ATGAGCGGAT	ACATATTGA	7260
	ATGTATTTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	AAGTGCCACC	7320
	TGACGTCGAC	GGATCGGGAG	ATCTGCTAGG	TGACCTGAGG	CGCGCCGGCT	TCGAATAGCC	7380
	AGAGTAACCT	TTTTTTTAA	TTTTATTTTA	TTTTATTTTT	GAGATGGAGT	TTGGCGCCGA	7440
5	TCTCCCGATC	CCCTATGGTC	GACTCTAGT	ACAATCTGCT	CTGATGCCGC	ATAGTTAAGC	7500
	CAGTATCTGC	TCCCTGCTTG	TGTGTTGGAG	GTCGCTGAGT	AGTGCGCGAG	CAAAAATTAA	7560
	GCTACAACAA	GGCAAGGCTT	GACCGACAAT	TGCATGAAGA	ATCTGCTTAG	GGTTAGGCGT	7620
	TTTGCGCTGC	TTGCGGATGT	ACGGGCCAGA	TATACGCGTT	GACATTGATT	ATTGACTAGT	7680
	TATTAATAGT	AATCAATTAC	GGGGTCATTA	GTTTCATAGC	CATATATGGA	GTTCCGCGTT	7740
10	ACATAACTTA	CGGTAAATGG	CCCGCCTGGC	TGACCGCCCA	ACGACCCCG	CCCATTGACG	7800
	TCAATAATGA	CGTAGTTTCC	CATAGTAACG	CCAATAGGGA	CTTCCATTG	ACGTCAATGG	7860
	GTGGACTATT	TACGGTAAAC	TGCCCACTTG	GCAGTACATC	AAGTGTATCA	TATGCCAAGT	7920
	ACGCCCCCTA	TTGACGTCAA	TGACGGTAAA	TGGCCCGCCT	GGCATTATGC	CCAGTACATG	7980
	ACCTTATGGG	ACTTTCCTAC	TTGGCAGTAC	ATCTACGTAT	TAGTCATCGC	TATTACCATG	8040
15	GTGATGCGGT	TTTGGCAGTA	CATCAATGGG	CGTGGATAGC	GGTTTGA	ACGGGGATT	8100
	CCAAGTCTCC	ACCCCAATTGA	CGTCAATGGG	AGTTTGT	GGCACAAAA	TCAACGGGAC	8160
	TTTCCAAAAT	GTCGTAACAA	CTCCGCCCCA	TTGACGCAAA	TGGGCGGTAG	GCGTGTACGG	8220
	TGGGAGGTCT	ATATAAGCAG	AGCTCTCTGG	CTAACTAGAG	AACCCACTGC	TTACTGGCTT	8280
20	ATCGAAATTA	ATACGACTCA	CTATAGGGAG	ACCCAAGCTT	G		8321

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8897 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

	GACGGATCGG	GAGATCTGCT	AGCCCGGGTG	ACCTGAGGCG	CGCCGGCTTC	GAATAGCCAG	60
	AGTAACCTTT	TTTTTTAATT	TTATTTTATT	TTATTTTGA	GATGGAGTTT	GGCGCCGATC	120
35	TCCCGATCCC	CTATGGTCGA	CTCTCAGTAC	AATCTGCTCT	GATGCCGCAT	AGTTAAGCCA	180
	GTATCTGCTC	CCTGCTTGTG	TGTTGGAGGT	CGCTGAGTAG	TGCGCGAGCA	AAATTTAAGC	240
	TACAACAAGG	CAAGGCTTGA	CCGACAATTG	CATGAAGAAT	CTGCTTAGGG	TTAGGCGTTT	300
	TGCGCTGCTT	CGCGATGTAC	GGGCCAGATA	TACGCGTTGA	CATTGATTAT	TGACTAGTTA	360
	TTAATAGTAA	TCAATTACGG	GGTCATTAGT	TCATAGCCCA	TATATGGAGT	TCCGCGTTAC	420
40	ATAACTTACG	GTAATGGCC	CGCCTGGCTG	ACCGCCCAAC	GACCCCGGCC	CATTGACGTC	480
	AATAATGACG	TATGTTCCCA	TAGTAACGCC	AATAGGGACT	TTCCATTGAC	GTCAATGGGT	540
	GGACTATTTA	CGGTAAACTG	CCCACTTGGC	AGTACATCAA	GTGTATCATA	TGCCAAGTAC	600
	GCCCCCTATT	GACGTCAATG	ACGGTAAATG	GCCCCGCTGG	CATTATGCCC	AGTACATGAC	660
	CTTATGGGAC	TTTCTACTT	GGCAGTACAT	CTACGTATTA	GTCATCGCTA	TTACCATGGT	720
45	GATGCGGTTT	TGGCAGTACA	TCAATGGGCG	TGGATAGCGG	TTTGACTCAC	GGGGATTTCC	780
	AAGTCTCCAC	CCCAATTGACG	TCAATGGGAG	TTTGT	TTTGG	CACCAAAATC	840
	TCCAAAATGT	CGTAACAAC	CCGCCCAT	GACGCAATG	GGCGGTAGGC	GTGTACGGTG	900
	GGAGGTCTAT	ATAAGCAGAG	CTCTCTGGCT	AACTAGAGAA	CCCACTGCTT	ACTGGCTTAT	960
	CGAAATTAAT	ACGACTCACT	ATAGGGAGAC	CCAAGCTTGG	TACCAATTTA	AATTGATATC	1020
50	TCCTTAGGTC	TGAGCACCA	TGAAGTTGCC	TGTTAGGCTG	TTGGTGCTGA	TGTTCTGGAT	1080
	TCCTGCTTCC	AGCAGTGATG	TTGTCATGAC	CCAAACCCCA	CTGTCCAGTC	CTGTCACGCT	1140
	TGGACAACCT	GCGTCCATCT	CTTGCGAGATC	TAGTCAGATC	ATTGTACATA	ATAATGGCAA	1200
	CACCTATCTG	GAATGGTACC	AGCAGAGACC	AGGGCAGTCT	CCACGGCTCC	TGATCTACAA	1260
	AGTTTCCAAC	CGATTTTCTG	GGGTCCCGA	CAGGTTTCAGC	GGCAGTGGAG	CTGGGACAGA	1320
55	TTTCACACTC	AAGATCAGCA	GAGTGGAGGC	TGAGGATGTG	GGAGTTTACT	ACTGCTTCCA	1380
	GGGTTACAT	GTTCCATCA	CGTTCGGCCA	AGGGACAAAG	TTGGAATCA	AACGTAAGTC	1440
	TCGAGTCTCT	AGATAACCGG	TCAATCGATT	GGAATTCTAA	ACTCTGAGGG	GGTCGGATGA	1500
	CGTGGCCATT	CTTTCCTTAA	AGCATTGAGT	TTACTGCAAG	GTCAGAAAAG	CATGCAAAGC	1560
	CCTCAGAATG	GCTGCAAAGA	GCTCCAACAA	AACAATTTAG	AACTTTATTA	AGGAATAGGG	1620

	GGAAGCTAGG	AAGAACTCA	AAACATCAAG	ATTTTAAATA	CGCTTCTTGG	TCTCCTTGCT	1680
	ATAATTATCT	GGGATAAGCA	TGCTGTTTTT	TGTCTGTCCC	TAACATGCCC	TTATCCGCAA	1740
	ACAACACACC	CAAGGGCAGA	ACTTTGTTAC	TTAAACACCA	TCCTGTTTGC	TTCTTTCTCT	1800
	AGGAACTGTG	GCTGCACCAT	CTGTCTTCAT	CTTCCCGCCA	TCTGATGAGC	AGTTGAAATC	1860
5	TGGAAGTGGC	TCTGTTGTGT	GCCTGCTGAA	TAACTTCTAT	CCCAGAGAGG	CCAAAGTACA	1920
	GTGGAAGGTG	GATAACGCCC	TCCAATCGGG	TAACCTCCAG	GAGAGTGTCA	CAGAGCAGGA	1980
	GAGCAAGGAC	AGCACCTACA	GCCTCAGCAG	CACCCTGACG	CTGAGCAAAG	CAGACTACGA	2040
	GAAACACAAA	GTCTACGCCT	GCGAAGTCAC	CCATCAGGGC	CTGAGCTCGC	CCGTACACAA	2100
	GAGCTTCAAC	AGGGGAGAGT	GTTAGAGGGA	GAAGTGCCCC	CACCTGCTCC	TCAGTTCCAG	2160
10	CCTGACCCCC	TCCCATCCTT	TGGCCTCTGA	CCCTTTTTC	ACAGGGGACC	TACCCCTATT	2220
	GCGGTCTCTC	AGCTCATCTT	TCACCTCACC	CCCCTCCTCC	TCCTTGGCTT	TAATTATGCT	2280
	AATGTTGGAG	GAGAATGAAT	AAATAAAGTG	AATCTTTGCA	CCTGTGGTTT	CTCTCTTTCC	2340
	TCATTTAATA	ATTATTATCT	GTTGTTTTAC	CAACTACTCA	ATTTCTCTTA	TAAGGGACTA	2400
	AATATGTAGT	CATCCTAAGG	CACGTAACCA	TTTATAAAAA	TCATCCTTCA	TTCTATTTTA	2460
15	CCCTATCATC	CTCTGCAAGA	CAGTCTCTCC	TCAAACCCAC	AAGCCTTCTG	TCCTCACAGT	2520
	CCCCTGGGCC	ATGGTAGGAG	AGACTTGCTT	CCTTGTTTTT	CCCTCCTCAG	CAAGCCCTCA	2580
	TAGTCTTTT	TAAGGGTGAC	AGGTCTTACA	GTCATATATC	CTTTGATTCA	ATTCCCTGAG	2640
	AATCAACCAA	AGCAAATTTT	TCAAAGAAG	AAACCTGCTA	TAAAGAGAAT	CATTCAATGC	2700
	AACATGATAT	AAAATAACAA	CACAATAAAA	GCAATTAAT	AAACAAACAA	TAGGGAAATG	2760
20	TTTAAGTTCA	TCATGGTACT	TAGACTTAAT	GGAATGTCAT	GCCTTATTTA	CATTTTTTAA	2820
	CAGGTACTGA	GGGACTCCTG	TCTGCCAAGG	GCCGTATTGA	GTACTTTCCA	CAACCTAATT	2880
	TAATCCACAC	TATACTGTGA	GATTAAAAAC	ATTCATTAAA	ATGTTGCAAA	GGTTCATATA	2940
	AGCTGAGAGA	CAAAATATAT	CTATAACTCA	GCAATCCAC	TTCTAGATGA	CTGAGTGTCC	3000
	CCACCCACCA	AAAAACTATG	CAAGAATGTT	CAAAGCAGCT	TTATTTACAA	AAGCCAAAAA	3060
25	TTGGAATAG	CCCGATTGTC	CAAGATTAGA	ATGAGTTAT	AAACTGTGGT	ATGTTTATAC	3120
	ATTAGAATAC	CCAATGAGGA	GAATTAACAA	GCTACAATA	TACCTACTCA	CACAGATGAA	3180
	TCTCATAAAA	ATAATGTTAC	ATAAGAGAAA	CTCAATGCAA	AAGATATGTT	CTGTATGTTT	3240
	TCATCCATAT	AAAGTTCAAA	ACCAGGTAAA	AATAAAGTTA	GAAATTTGGA	TGGAAATTAC	3300
	TCTTAGCTGG	GGGTGGGCGA	GTTAGTGCTT	GGGAGAAGAC	AAGAAGGGGC	TTCTGGGGTC	3360
30	TTGGTAATGT	TCTGTTCTCT	GTGTGGGGTT	GTGCAGTTAT	GATCTGTGCA	CTGTCTGTGA	3420
	TACACATTAT	GCTTCAAAAT	AACTTCACAT	AAAGAACATC	TTATACCCAG	TTAATAGATA	3480
	GAAGAGGAAT	AAGTAATAGG	TCAAGACCAA	CGCAGCTGGT	AAGTGGGGGC	CTGGGATCAA	3540
	ATAGCTACCT	GCCTAATCCT	GCCCWCTTGA	GCCCTGAATG	AGTCTGCCTT	CCAGGGCTCA	3600
	AGGTGCTCAA	CAAAACAACA	GGCCTGCTAT	TTTCCTGGCA	TCTGTGCCCT	GTTTGGCTAG	3660
35	CTAGGAGCAC	ACATACATAG	AAATTTAAATG	AAACAGACCT	TCAGCAAGGG	GACAGAGGAC	3720
	AGAATTAACC	TTGCCCAGAC	ACTGGAACCC	CATGTATGAA	CACTCACATG	TTTGGGAAGG	3780
	GGGAAGGGCA	CATGTAATATG	AGGACTCTTC	CTCATTCTAT	GGGGCACTCT	GGCCCTGCCC	3840
	CTCTCAGCTA	CTCATCCATC	CAACACACCT	TTCTAAGTAC	CTCTCTCTGC	CTACACTCTG	3900
	AAGGGGTTCA	GGAGTAACTA	ACACAGCATC	CCTTCCCTCA	AATGACTGAC	AATCCCTTTG	3960
40	TCCTGCTTTG	TTTTTCTTTC	CAGTCAGTAC	TGGGAAAGTG	GGGAAGGACA	GTCATGGAGA	4020
	AACTACATAA	GGAAGCACCT	TGCCCTTCTG	CCTCTTGAGA	ATGTTGATGA	GTATCAAATC	4080
	TTTCAAACCT	TGGAGGTTTG	AGTAGGGGTG	AGACTCAGTA	ATGTCCCTTC	CAATGACATG	4140
	AACTTGCTCA	CTCATCCCTG	GGGGCCAAAT	TGAACAATCA	AAGGCAGGCA	TAATCCAGTT	4200
	ATGAATCTT	GCGGCCGCTT	GCTAGCTTCA	CGTGTGGGAT	CCAACCGCGG	AAGGGCCCTA	4260
45	TTCTATAGTG	TCACCTAAAT	GCTAGAGCTC	GCTGATCAGC	CTCGACTGTG	CCTTCTAGTT	4320
	GCCAGCCATC	TGTTGTTTGC	CCCTCCCCCG	TGCCTTCCTT	GACCCTGGAA	GGTGCCACTC	4380
	CCACTGTCTT	TTCTTAATAA	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCATT	4440
	CTATTCTGGG	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA	GACAATAGCA	4500
	GGCATGCTGG	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAACC	AGCTGGGGCT	4560
50	CTAGGGGGTA	TCCCCACGCG	CCCTGTAGCG	GCGCATTAAG	CGCGGCGGGT	GTGGTGGTTA	4620
	CGCGCAGCGT	GACCGCTACA	CTTGCCAGCG	CCCTAGCGCC	CGCTCCTTTC	GCTTCTTCTC	4680
	CTTCTTTTCT	CGCCACGTTT	GCCGGGCCTC	TCAAAAAAGG	GAAAAAAAGC	ATGCATCTCA	4740
	ATTAGTCAGC	AACCATAGTC	CCGCCCCCTA	CTCCGCCCCAT	CCCGCCCCCTA	ACTCCGCCCA	4800
	GTTCCGCCCA	TTCTCCGCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA	GAGGCCGAGG	4860
55	CCGCCTCGGC	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG	CTTTTTTGGA	GGCCTAGGCT	4920
	TTTGCAAAAA	GCTTGGACAG	CTCAGGGCTG	CGATTTGCGG	CCAAACTTGA	CGGCAATCCT	4980
	AGCGTGAAGG	CTGGTAGGAT	TTTATCCCCG	CTGCCATCAT	GGTTCGACCA	TTGAACTGCA	5040
	TCGTGCGCGT	GTCCCAAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC	5100
	TCAGGAACGA	GTTCAAGTAC	TTCCAAGAA	TGACCACAAC	CTCTTCAGTG	GAAGGTAAAC	5160

	AGAATCTGGT	GATTATGGGT	AGGAAAACCT	GGTCTCCAT	TCCTGAGAAG	AATCGACCTT	5220
	TAAAGGACAG	AATTAATATA	GTTCTCAGTA	GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	5280
	ATTTTCTTGC	CAAAAGTTTG	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	5340
	GTAAAGTAGA	CATGGTTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAGGAA	GCCATGAATC	5400
5	AACCAGGCCA	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAATTTGAA	AGTGACACGT	5460
	TTTTCCCAGA	AATTGATTTG	GGGAAATATA	AACTTCTCCC	AGAATACCCA	GGCGTCTCT	5520
	CTGAGGTCCA	GGAGGAAAAA	GGCATCAAGT	ATAAGTTTGA	AGTCTACGAG	AAGAAAGACT	5580
	AACAGGAAGA	TGCTTTCAAG	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	5640
	CATGGGACTT	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC	5700
10	ATAATTGGAC	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAAATATAA	AATTTTAAAG	5760
	TGTATAATGT	GTTAAACTAC	TGATTCTAAT	TGTTTGTGTA	TTTTAGATTG	CAACCTATGG	5820
	AACTGATGAA	TGGGAGCAGT	GGTGAATGTC	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	5880
	AGAAATGCCA	TCTAGTGATG	ATGAGGCTAC	TGCTGACTCT	CAACATCTTA	CTCCTCCAAA	5940
	AAAGAAGAGA	AAGGTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCTAA	GTTTTTTGAG	6000
15	TCATGCTGTG	TTTAGTAATA	GAACCTTTGC	TTGCTTTGCT	ATTTACACCA	CAAAGGAAAA	6060
	AGCTGCACTG	CTATACAAGA	AAATTATGGA	AAAATATTCT	GTAACCTTTA	TAAGTAGGCA	6120
	TAACAGTTAT	GTAAACTACA	TACTGTTTTT	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	6180
	TATTAATAAT	TATGCTCAAA	AATTGTGTAC	CTTTAGCTTT	TTAATTTGTA	AAGGGGTAA	6240
	TAAGGAATAT	TTGATGTATA	GTGCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTG	6300
20	TAGAGGTTTT	ACTTGCTTTA	AAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA	6360
	TGAATGCAAT	TGTTGTTGTT	AACTTGTTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAAGCA	6420
	ATAGCATCAC	AAATTTTACA	AATAAAGCAT	TTTTTTTCACT	GCATTCTAGT	TGTGGTTTGT	6480
	CCAAACTCAT	CAATGTATCT	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	6540
	ATCTCATGCT	GGAGTTCTTC	GCCCCACCCA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA	6600
25	AATAAAGCAA	TAGCATCACA	AATTTTCAAA	ATAAAGCATT	TTTTTCACTG	CATTCTAGTT	6660
	GTGGTTTGTG	CAAACTCATC	AATGTATCTT	ATCATGTCTG	TATACCGTCG	ACCTCTAGCT	6720
	AGAGCTTGGC	GTAAATCATGG	TCATAGCTGT	TTCTGTGTG	AAATTGTTAT	CCGCTCACAA	6780
	TTCCACACAA	CATACGAGCC	GGAAGCATAA	AGTGTAAAGC	CTGGGGTGCC	TAATGAGTGA	6840
	GCTAACTCAC	ATTAATTGCG	TTGCGCTCAC	TGCCCGCTTT	CCAGTCGGGA	AACCTGTCGT	6900
30	GCCAGCTGCA	TTAATGAATC	GGCCAAACCG	CGGGGAGAGG	CGGTTTGCGT	ATTGGGCGCT	6960
	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	GCTCGGTCTG	TCCGCTGCGG	CGAGCGGTAT	7020
	CAGCTCACTC	AAAGGCGGTA	ATACGGTTAT	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	7080
	ACATGTGAGC	AAAAGGCCAG	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	7140
	TTTTCCATAG	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT	7200
35	GGCGAAACCC	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC	TCCCTCGTGC	7260
	GCTCTCCTGT	TCCGACCCTG	CCGCTTACCG	GATACCTGTG	CGCCTTTCTC	CCTTCGGGAA	7320
	GCGTGGCGCT	TTCTCAATGC	TCACGCTGTA	GGTATCTCAG	TTCCGGTGTAG	GTCGTTGCGT	7380
	CCAAGCTGGG	CTGTGTGCAC	GAACCCCCCG	TTCAGCCCCG	CCGCTGCGCC	TTATCCGGTA	7440
	ACTATCGTCT	TGAGTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG	7500
40	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC	7560
	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG	AAGCCAGTTA	7620
	CCTTCGGAAG	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAAACA	AACCAACCGT	GGTAGCGGTG	7680
	GTTTTTTTGT	TTGCAAGCAG	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	7740
	TGATCTTTTC	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	7800
45	TCATGAGATT	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATTAAAAA	TGAAGTTTTA	7860
	AATCAATCTA	AAGTATATAT	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	7920
	AGGCACCTAT	CTCAGCGATC	TGTCTATTTT	GTTTCATCCAT	AGTTGCCTGA	CTCCCCGTCG	7980
	TGTAGATAAC	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA	ATGATACCGC	8040
50	GAGACCCACG	CTCACC GGCT	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGAAGGGCCG	8100
	AGCGCAGAAG	TGGTCTTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	8160
	AAGCTAGAGT	AAGTAGTTTC	CCAGTTAATA	GTTTGC GCAA	CGTTGTTGCC	ATTGTACAG	8220
	GCATCGTGGT	GTCACGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	8280
	CAAGGCGAGT	TACATGATCC	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC	TTCCGTCCTC	8340
	CGATCGTTGT	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC	8400
55	ATAATTCTCT	TACTGTCATG	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	8460
	CCAAGTCATT	CTGAGAAATG	TGTATGCGGC	GACCGAGTTG	CTCTTGCCCG	GCGTCAATAC	8520
	GGGATAATAC	CGCGCCACAT	AGCAGAACTT	TAAAAGTGCT	CATCATTTGA	AAACGTTCTT	8580
	CGGGGCGAAA	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTCCGATG	TAACCCACTC	8640
	GTGCACCCAA	CTGATCTTCA	GCATCTTTTA	CTTTCACCAG	CGTTTCTGGG	TGAGCAAAAA	8700

CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	8760
TACTCTTCCT	TTTTCAATAT	TATTGAAGCA	TTATCAGGG	TTATTGTCTC	ATGAGCGGAT	8820
ACATATTGGA	ATGTATTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTCCCCGAA	8880
AAGTGCCACC	TGACGTC					8897

What is claimed is:

1. A method for inhibiting immunoglobulin-induced toxicity resulting from
5 immunoglobulin immunotherapy in a subject comprising administering an
immunoglobulin molecule to the subject, the immunoglobulin molecule
having a variable region and a constant region, the immunoglobulin molecule
being modified prior to administration by structurally altering multiple
toxicity associated domains in the constant region so that immunoglobulin-
10 induced toxicity is inhibited.
2. A method for inhibiting immunoglobulin-induced toxicity resulting from
immunoglobulin immunotherapy in a subject comprising administering a
15 structurally altered antibody to the subject, the structurally altered antibody
comprising a variable region and a constant region, multiple toxicity
associated domains in the constant region being modified so as to render the
constant region unable to mediate an ADCC response or activate
complement thereby inhibiting immunoglobulin-induced toxicity resulting
from immunotherapy.
- 20 3. A method for inhibiting immunoglobulin-induced toxicity resulting from
immunotherapy in a subject comprising administering an Ig fusion protein to
the subject, the Ig fusion protein having multiple structurally altered toxicity
associated domains in the constant region.
- 25 4. A method for inhibiting immunoglobulin-induced toxicity resulting from
immunotherapy in a subject comprising administering an Ig fusion protein to
the subject, the Ig fusion protein comprising a modified constant region, the

modification being a structural alteration in multiple toxicity associated regions within the CH₂ domain.

5. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
 - (a) selecting an immunoglobulin which recognizes and binds a target, the target being associated with the disease;
 - (b) mutating the immunoglobulin so selected by structurally altering multiple toxicity associated domains in the constant region of the immunoglobulin thereby creating a structurally altered immunoglobulin;
 - (c) administering the structurally altered immunoglobulin of step (b) to the subject under conditions so that the structurally altered immunoglobulin recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the constant region thereby preventing immunoglobulin-induced toxicity in the subject.
6. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
 - (a) selecting an Ig fusion protein which recognizes and binds a target, the target being associated with the disease;
 - (b) structurally altering multiple toxicity associated domains in the CH₂ domain of the constant region of the Ig protein so selected;

- (c) administering the structurally altered Ig fusion protein of step (b) to the subject under conditions so that the structurally altered Ig fusion protein recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the CH₂ domain thereby preventing immunoglobulin-induced toxicity in the subject.
- 5
7. The method of claim 1, 2, 3, 4, 5, or 6, wherein the portion of the constant region is the CH₂ domain.
- 10
8. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgG.
9. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgM.
- 15
10. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgA.
11. The method of claim 2, wherein the antibody recognizes and binds Le^y.
12. The method of claim 2, wherein the antibody recognizes and binds to Le^x.
- 20
13. The method of claim 2, wherein the antibody is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
- 25
14. The method of claim 2, wherein the antibody is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.

15. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds Le^y.
16. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds to Le^x.
17. The method of claim 1 or 5, wherein the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
18. The method of claim 1 or 5, wherein the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
19. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds Le^y.
20. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds to Le^x.
21. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
22. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
23. A pharmaceutical composition comprising a pharmaceutically effective

amount of a structurally altered immunoglobulin, and an acceptable carrier, the structurally altered immunoglobulin (1) recognizes and binds a target. the target is associated with cancer and (2) has an inactivated CH₂ domain.

- 5 24. A pharmaceutical composition comprising a pharmaceutically effective amount of structurally altered Ig fusion protein, and an acceptable carrier, the structurally altered Ig fusion protein (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.
- 10 25. A method of treating carcinomas in vivo comprising administering to a subject a pharmaceutically effective amount of the composition of claim 23 or 24.
- 15 26. The method of claim 30, wherein the structurally altered immunoglobulin in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.
- 20 27. The method of claim 24, wherein the Ig fusion protein in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.
- 25 28. The method of claim 2 or 5, wherein the antibody is conjugated to a cytotoxic agent.
29. The method of claim 1, wherein the immunoglobulin is conjugated to a cytotoxic agent.

30. The method of claim 3, 4 or 6, wherein the Ig fusion protein is conjugated to a cytotoxic agent.
31. The method of claim 28, 29, or 31, wherein the cytotoxic agent is selected
5 from the group consisting of antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents.
32. A method for treating a subject suffering from a cancer, the cancer being
10 characterized as a group of cells having a tumor associated antigen on the cell surface, which method comprises administering to the subject a cancer killing amount of the composition of claim 23 or 24 joined to a cytotoxic agent under conditions which permit the molecule so joined to bind the tumor associated antigen on the cell surface so as to kill the cells so bound
15 thereby curing the subject.
33. A pharmaceutical composition comprising a pharmaceutically effective amount of a structurally altered BR96 antibody, the structurally altered antibody having an inactivated CH₂ domain.
20
34. A method for treating a subject suffering from a proliferative type disease characterized by cells having a BR96 antigen on the cell surface which comprises administering to the subject an effective amount of the composition of claim 33 joined to doxorubicin such that the
25 immunoconjugate binds the BR96 antigen and kills said cells thereby treating the subject.
35. A method for inhibiting BR96 (ATCC: HB10036) induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering

BR96 to the subject, the BR96 molecule being modified prior to administration, the modification comprising the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated toxicity is inhibited.

36. A method for preventing BR96 (ATCC: HB10036) induced toxicity resulting from immunotherapy for cancer in a subject comprising:

(a) mutating the BR96 polypeptide by the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated immunoglobulin-induced toxicity is inhibited in the altered BR96 polypeptide; and

(b) administering the structurally altered BR96 polypeptide of step (a) to the subject under conditions so that the peptide recognizes and binds cancer associated Le^y antigens, thereby alleviating symptoms associated with the cancer, the structural alteration of the toxicity associated domains thereby preventing BR96 toxicity in the subject.

37. A chimeric BR96 antibody having a structurally altered constant region having the CH1 and CH3 domains but not the CH2 domain, the antibody being designated cBR96-A.

38. The chimeric BR96 antibody of claim 37 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 10.
39. A BR96 antibody having humanized variable and constant regions, wherein the constant region has been structurally altered so that the CH1 and CH3 domains are present but the CH2 domain is not, the antibody being designated hBR96-2A.
40. The BR96 antibody of claim 39 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 12.
41. A BR96 antibody designated hBR96-2B having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine and glycine at amino acid position 237 is mutated to alanine.
42. A BR96 antibody designated hBR96-2C having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.
43. A BR96 antibody designated hBR96-2D having a structurally altered constant region wherein proline at amino acid position 331 is mutated to alanine.
44. A BR96 antibody designated hBR96-2E having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid

position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.

45. A BR96 antibody designated hBR96-2F having a structurally altered
5 constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; and proline at amino acid position 331 is mutated to alanine.
46. A BR96 antibody designated hBR96-2G having a structurally altered
10 constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
47. A BR96 antibody designated hBR96-2H having a structurally altered
15 constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; lysine at amino acid position 322 is
20 mutated to serine; and proline at amino acid position 331 is mutated to alanine.
48. A nucleic acid molecule which encodes the BR96 antibody of claim 37, 39, and 41-47.
25
49. A cDNA of claim 48.
50. A plasmid which comprises the nucleic acid molecule of claim 48.

51. A host vector system comprising a plasmid of claim 50 in a suitable host cell.
52. A method for producing a protein comprising growing the host vector system of claim 51 so as to produce the protein in the host and recovering the protein so produced.
- 5

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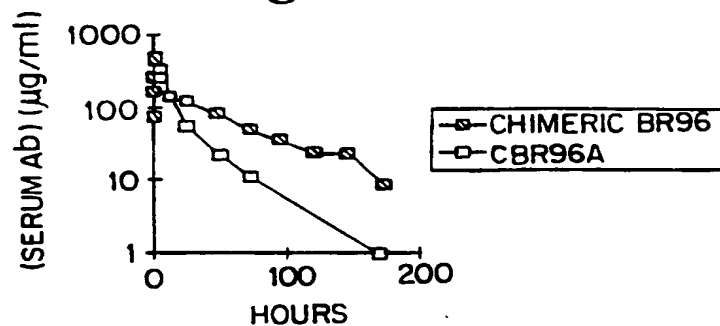
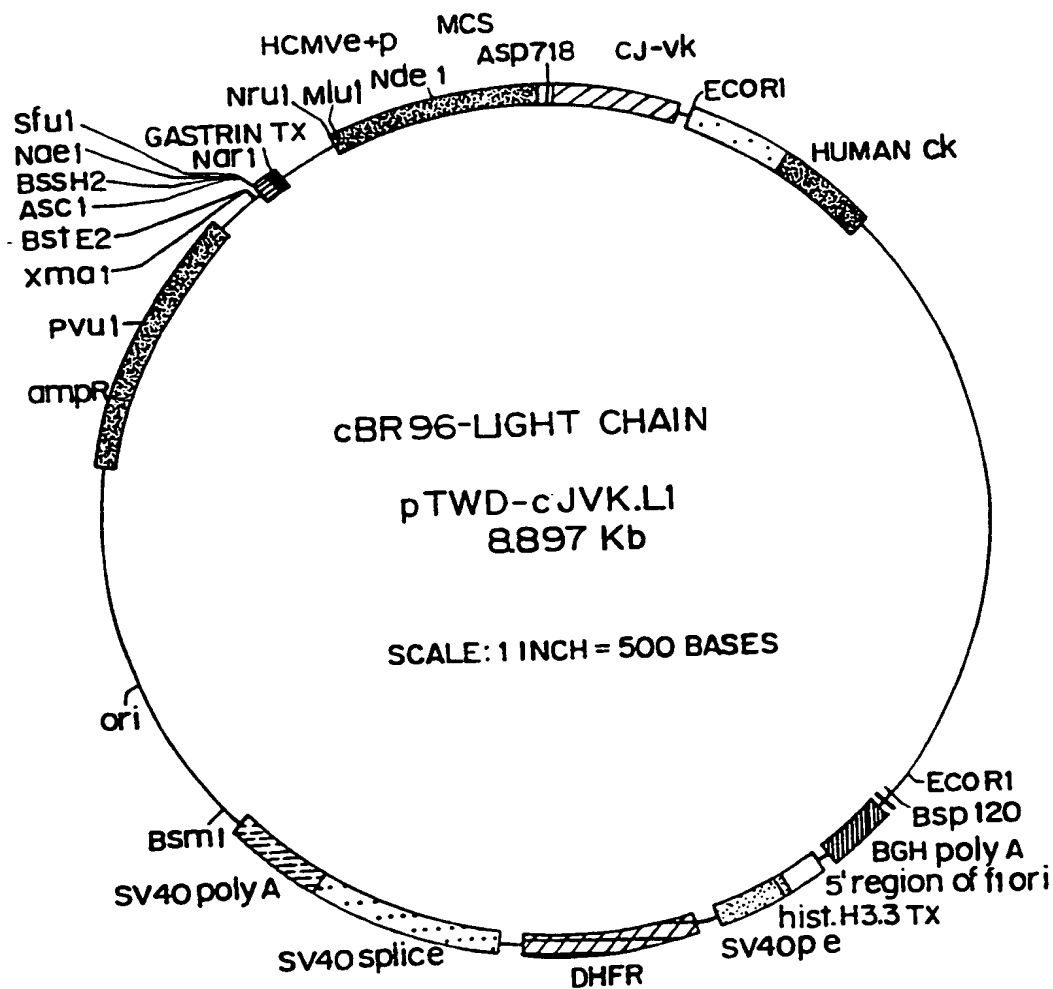
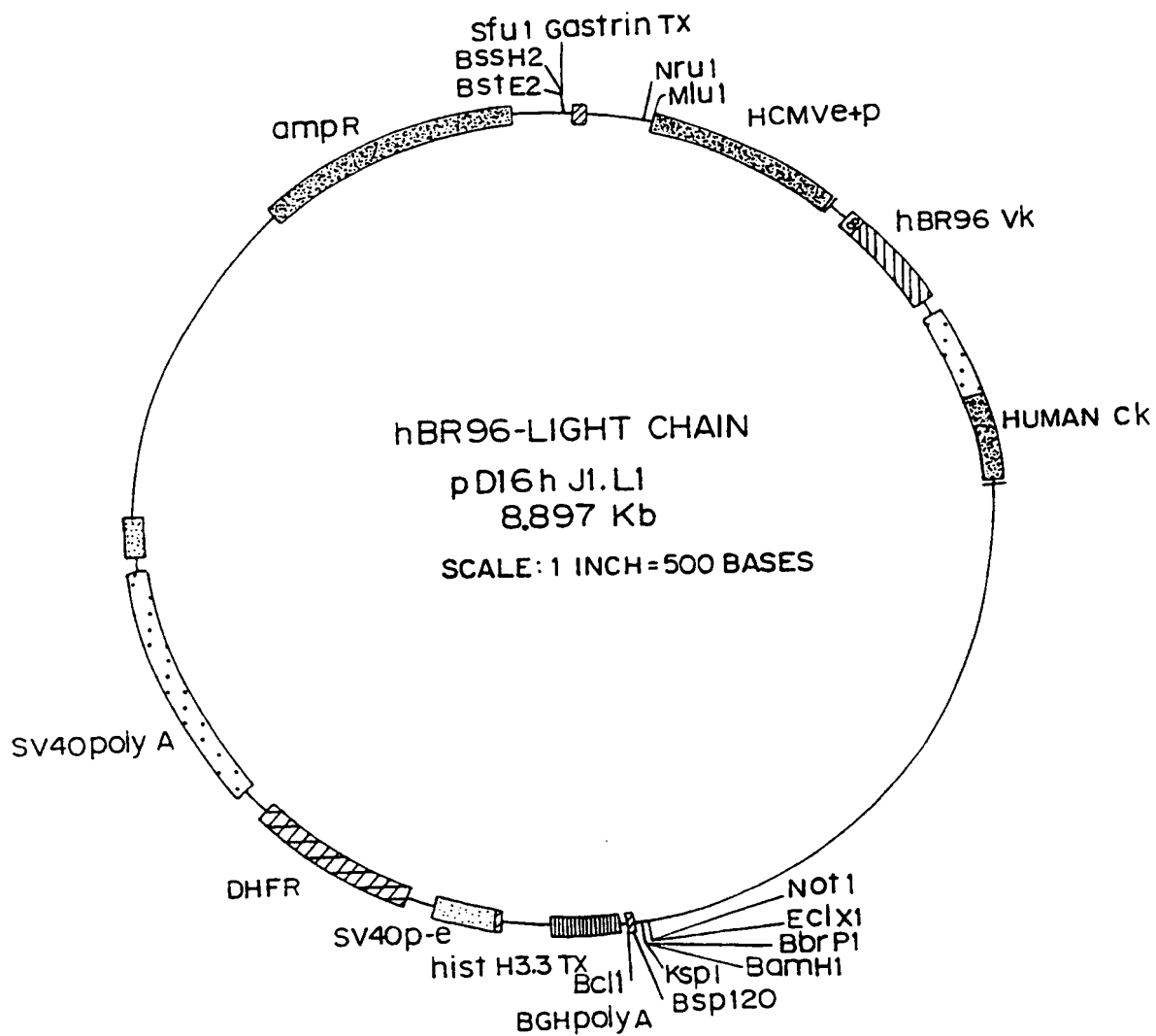
Fig. 1*Fig. 2*

Fig. 3

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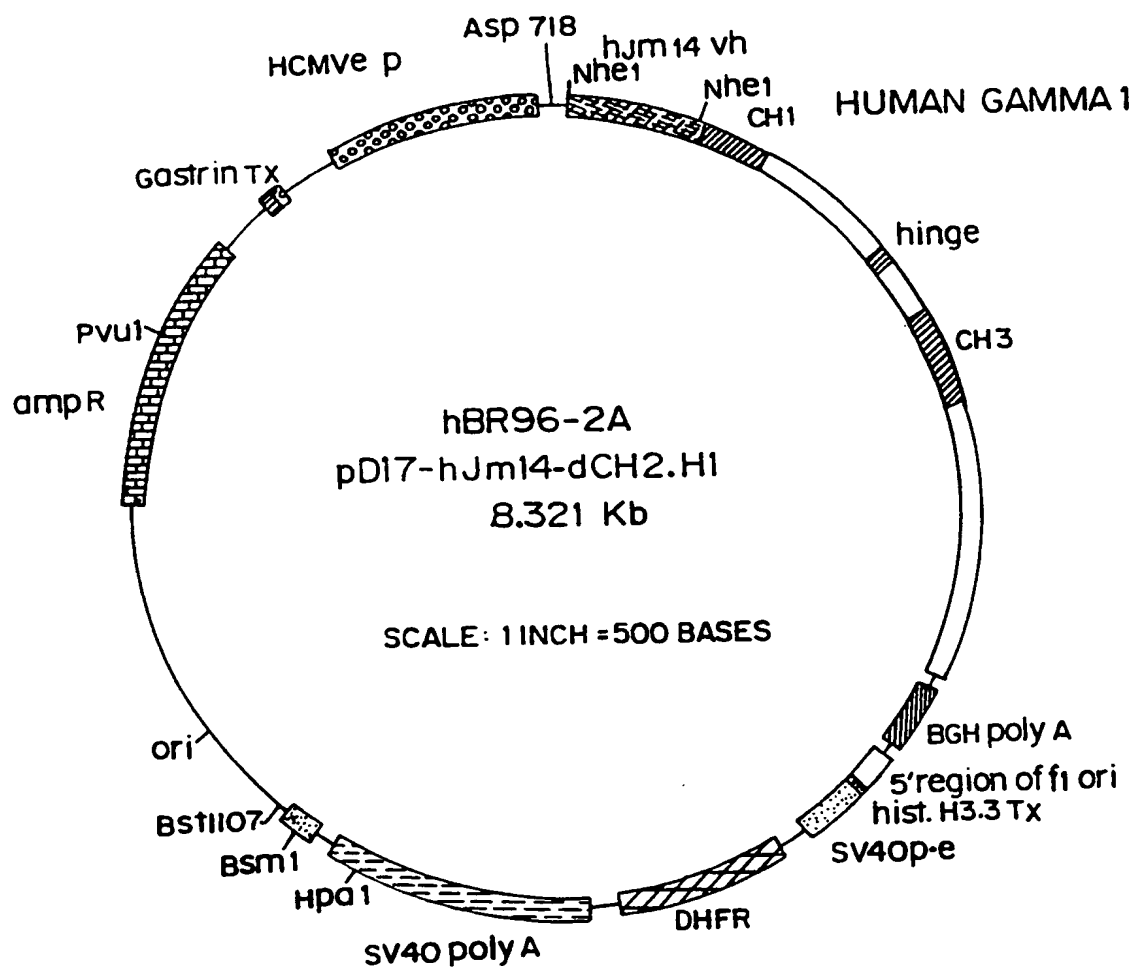
Fig. 4

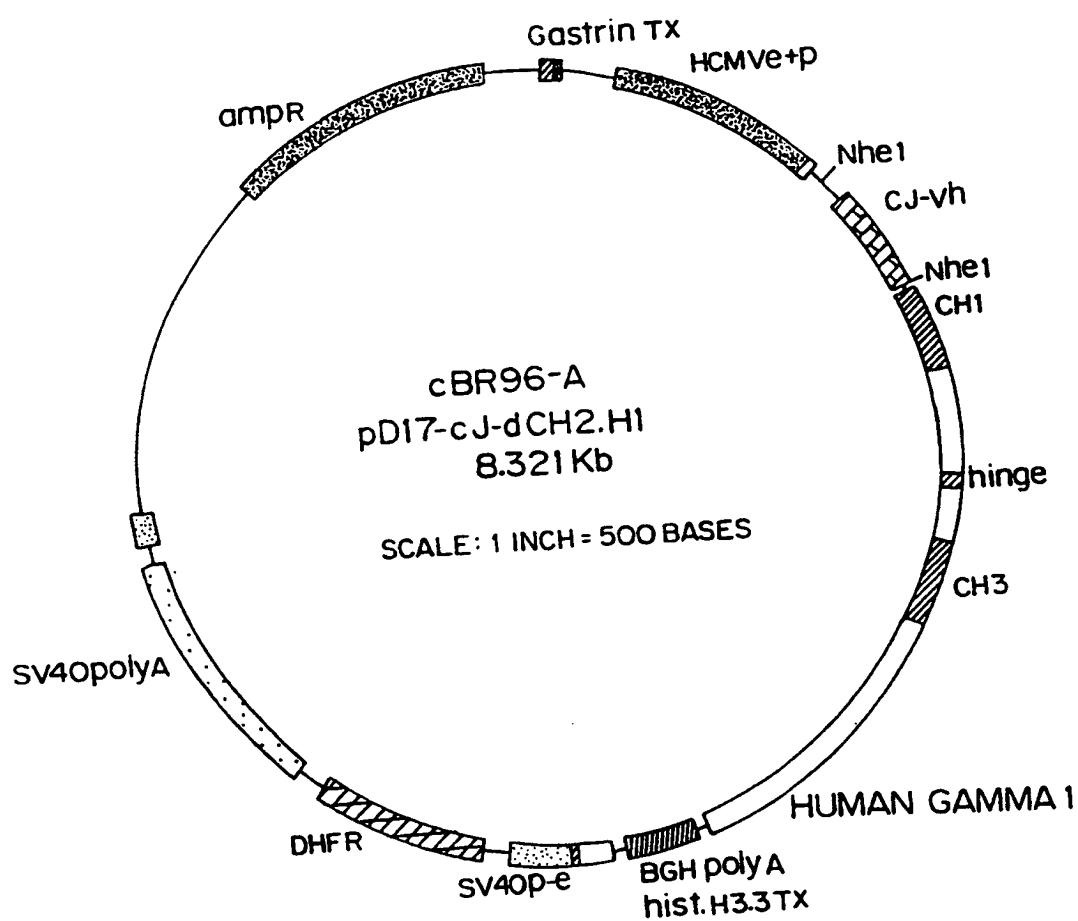
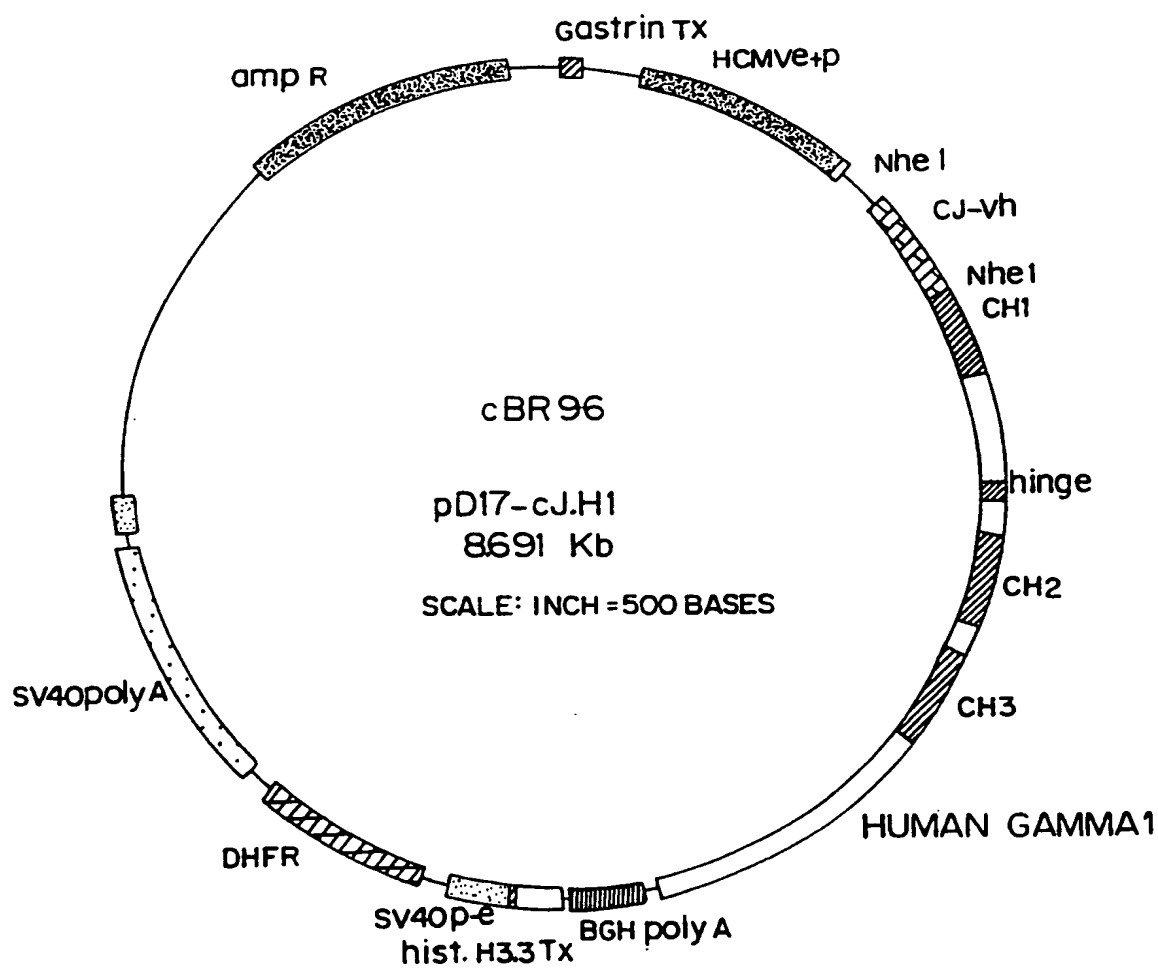
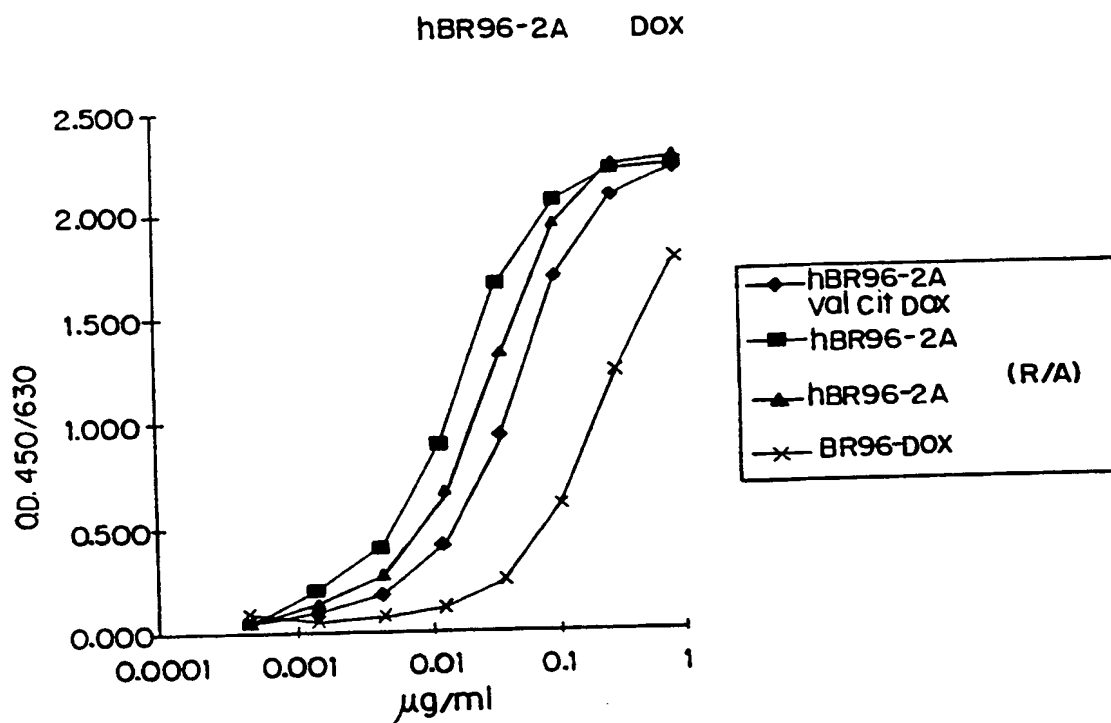
Fig. 5

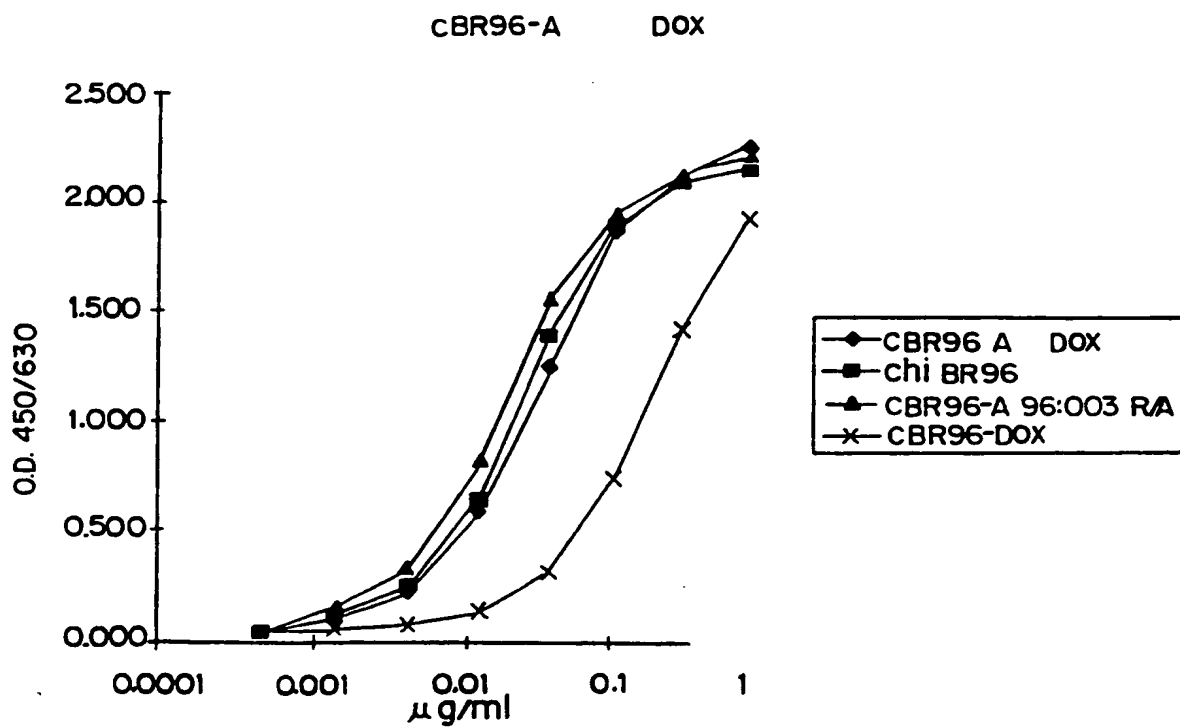
Fig. 6



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Fig. 7

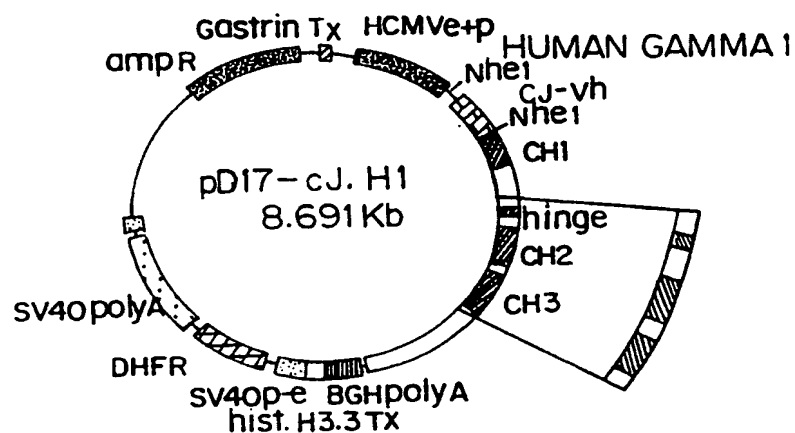
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Fig. 8

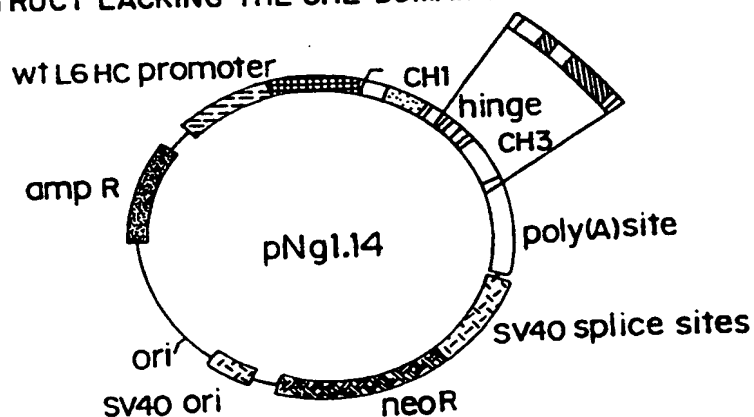
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Fig. 9A

A-HINGE + CH2+CH3 DOMAINS WERE REMOVED FROM BR96 IGG1
CONSTRUCT BY E.CO.47-III RESTRICTION DIGESTION.

**Fig. 9B**

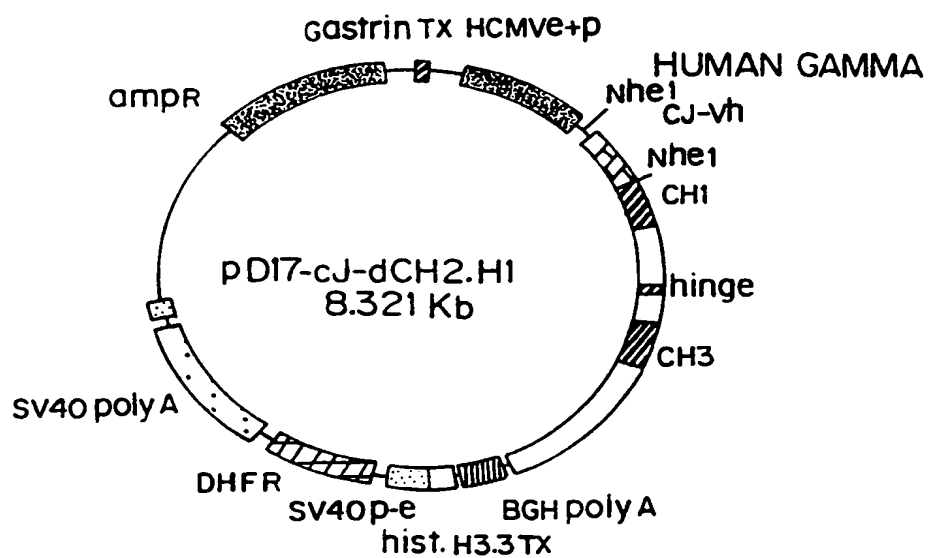
B-HINGE+CH3 DOMAINS AMPLIFIED BY PCR FROM L6 IGG1
CONSTRUCT LACKING THE CH2 DOMAIN.



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Fig. 9C

C-HINGE+CH3 PCR FRAGMENT CLONED BY HOMOLOGOUS
RECOMBINATION INTO E.CO.47-III SITE OF BR96 IGGI MOLECULE.



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1.- INTRODUCTION OF MUTATIONS BY SITE DIRECTED
MUTAGENESIS ON DOUBLE-STRANDED PLASMID DNA.

Fig. 10A

A.- MUTATIONS INTRODUCED INTO SYNTHETIC OLIGONUCLEOTIDES
USED FOR THE PCR AMPLIFICATION OF CH2 DOMAIN

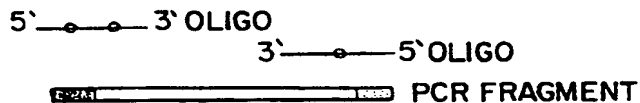


Fig. 10B

B.- PLASMID DNA LINEARIZED INSIDE CH2 DOMAIN AND CO-
TRANSFORMED WITH PCR FRAGMENT INTO COMPETENT DH5 α

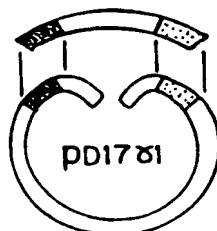
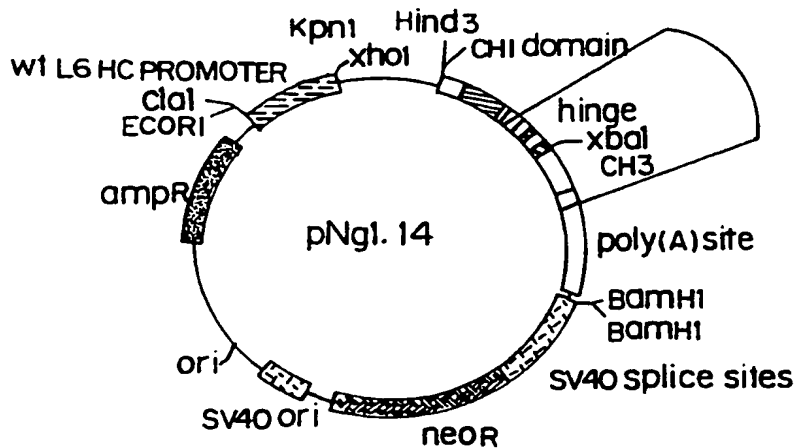
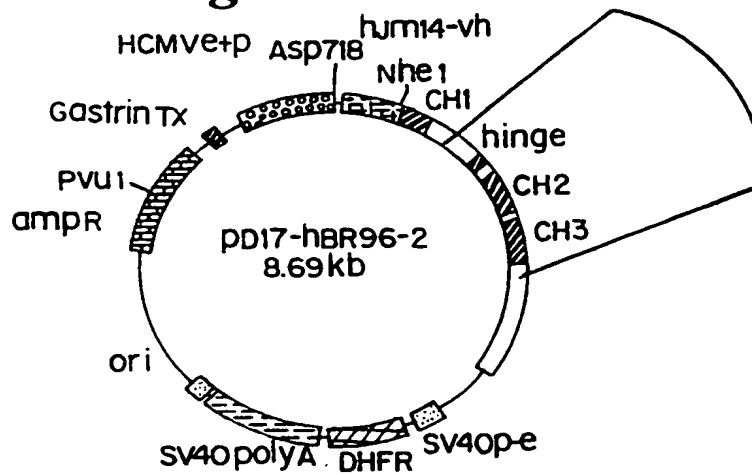
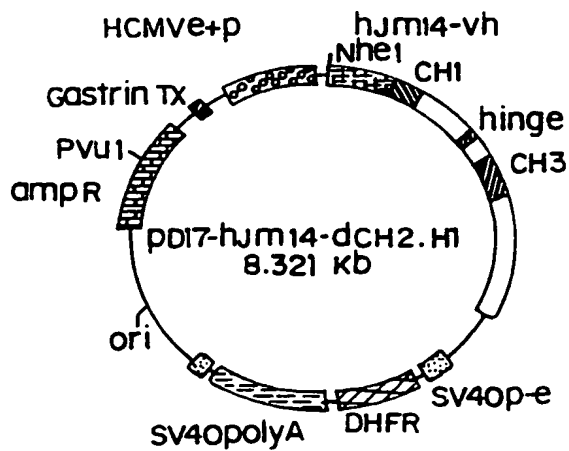


Fig. 10C

C.- CLONING MEDIATED BY HOMOLOGOUS RECOMBINATION YIELDS
TRANSFORMANTS HARBOURING RECOMBINANT PLASMIDS.



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Fig. 11**Fig. 12****Fig. 13**

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FIG. 14A

pd17-cJ-dCH2.H1

10	20	30	40	50	60	70	80	90
GACGGATCGG	GAGATCTGCT	AGGTGACCTG	AGGCGCGCGG	GCTTCGAATA	GCCAGAGTAA	CCTTTTITTT	TAATTTTATT	TTATTTTATT
CTGCCTAGCC	CTCTAGACGA	TCCACTGGAC	TCCGCGCGGC	CGAAGCTTAT	CGGTCTCAT	GGAAAAAA	AAAAATAA	AATAAAATAA
100	110	120	130	140	150	160	170	180
TTTGAGATGG	AGTTTGGCG	CGATCTCCG	ATCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC
AAACTCTACC	TCAAACCGCG	GCTAGAGGGC	TAGGGGATAC	CAGCTGAGAG	TCATGTTAGA	CGAGACTACG	CGGTATCAAT	TCGGTCATAG
190	200	210	220	230	240	250	260	270
TGCTCCCTGC	TTGTGTGTTG	GAGGTGCTG	AGTAGTGGC	GAGCAAAATT	TAAGCTACAA	CAAGGCAAGG	CTTGACCGAC	AAATTGCATGA
ACGAGGGACG	AACACACAAC	CTCCAGCGAC	TCATCAGCG	CTCGTTTAA	ATTCGATGTT	GTTCCGTTCC	GAACTGGCTG	TTAACGTAAT
280	290	300	310	320	330	340	350	360
AGAACTCTGCT	TAGGGTTAGG	CGTTTGGCG	TGCTTCGCGA	TGTACGGGC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAAAT
TCTTAGACGA	ATCCCAATCC	GCAAAACGCG	ACGAAGCGCT	ACATGCCCGG	TCTATATGCG	CAACTGTAACT	TAATAACTGA	TCAATAATTA
370	380	390	400	410	420	430	440	450
AGTAATCAAT	TACGGGTCA	TTAGTTTATA	GCCCATATAT	GGAGTTCGCG	GTTACATAAC	TTACGGTAAA	TGGCCCGCCT	GGCTGACCGC
TCAATTAGTTA	ATGCCCCAGT	AATCAAGTAT	CGGGTATATA	CCTCAAGGCG	CAATGTATTG	AATGCCATTT	ACCGGGCGGA	CCGACTGGCG
460	470	480	490	500	510	520	530	540
CCTAACGACC	CGGCCCATG	ACGTCAATAA	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT
GGTTGTCTGG	GGCGGGTAAC	TGCAGTTATT	ACTGCATACA	AGGGTATCAT	TGCGGTTATC	CCTGAAAGGT	AACTGCAGTT	ACCCACCTGA
550	560	570	580	590	600	610	620	630
ATTTACGGTA	AACTGCCAC	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	CTATTGACGT	CAATGACGGT	AAATGGCCCC
TAAATGCCAT	TTGACGGGTG	AACCGTCATG	TAGTTTCACAT	AGTATACGGT	TCATGCGGG	GATAACTGCA	GTTACTGCCA	TTTACCGGGC
640	650	660	670	680	690	700	710	720
CCTGGCATTA	TGCCCAGTAC	ATGACCTTAT	GGGACTTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC
GGACCGTAAT	ACGGGTCATG	TACTTGAATA	CCCTGAAAGG	ATGAACCGTC	ATGTAGATGC	ATAATCAGTA	CGGATAATGG	TACCACCTAG
730	740	750	760	770	780	790	800	810
GGTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCAGGGGA	TTTCCAAGTC	TCCACCCCAT	TGACGTCAT	GGGAGTTTGT
CCAAAACCGT	CATGTAGTTA	CCCGCACCTA	TGCGCAAACT	GAGTGCCCT	AAAGGTTTCA	AGGTGGGTA	ACTGCAGTTA	CCCTCAAAACA
820	830	840	850	860	870	880	890	900
TTTGGCACCA	AAATCAACGG	GACTTTCCAA	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGCGG	TAGGCGTGTA	CGGTGGGAGG
AAACCGTGGT	TTTAGTTGCC	CTGAAAGGTT	TTACAGCAT	GTTAGGCGG	GGTAACTGCG	TTTACCCGCC	ATCCGCACAT	GCCACCCCTCC

FIG. 14B

PD17-cJ-dCH2.H1

910	920	930	940	950	960	970	980	990
TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	TTAATACGAC	TCACTATAGG	GAGACCCAAG
AGATATATTC	GTCTCGAGAG	ACCGATTGAT	CTCTTGGGTG	ACGAATGACC	GAATAGCTTT	AATTATGCTG	AGTGATATCC	CTCTGGGTTC
1000	1010	1020	1030	1040	1050	1060	1070	1080
CTTGGTACCA	ATTAAATTG	ATACTCCTT	AGGCTCGAG	TCTCTAGATA	ACCGTCAAT	CGATTGGAAT	TCTTGGGGCC	GCTTGCTAGC
GAACCATGGT	TAAATTAAAC	TATAGAGGAA	TCCAGAGCTC	AGAGATCTAT	TGGCCAGTTA	GCTAACCTTA	AGAACGCCGG	CGAACGATCG
1090	1100	1110	1120	1130	1140	1150	1160	1170
CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCTT	TGTTTTAAAA	GGTGTCAGT	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG
GTGGTACCCT	AACACCAATT	CGAACCCAGGA	AGGNACAGGA	ACAAAATTTT	CCACAGGTCA	CACCTCACTT	AGACCACCTC	AGACCCCTC
1180	1190	1200	1210	1220	1230	1240	1250	1260
GCTTAGTGCA	GCCTGGAGG	TCCCTGAAG	TCTCCTGTGT	AACCTCTGGA	TTCACCTTCA	GTGACTATTA	CATGTATTGG	GTTGCGCCAGA
CGAATCACGT	CGGACCTCCC	AGGGACTTTC	AGAGGACACA	TTGGAGACCT	AAGTGAAAGT	CACCTGATAAT	GTACATAAAC	CAAGCGGTCT
1270	1280	1290	1300	1310	1320	1330	1340	1350
CTCCAGAGAA	GAGGCTGGAG	TGGGTGCGAT	ACATTAGTCA	AGGTGGTGAT	ATAACCGACT	ATCCAGACAC	TGTAAAGGGT	CGATTTCACCA
GAGGTCTCTT	CTCCGACCTC	ACCCAGCGTA	TGTAATCAGT	TCCACCACTA	TATTGGCTGA	TAGGTCTGTG	ACATTTCCTCA	GCTTAAGTGGT
1360	1370	1380	1390	1400	1410	1420	1430	1440
TCTCCAGAGA	CAATGCCAAG	AACACCCTGT	ACCTGCAAAAT	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC
AGAGGTCTCT	GTTACGGTTC	TTGTGGGACA	TGGACGTTTA	CTCGGCAGAC	TTCAGACTCC	TGTGTGCGTA	CATAATGACA	CGTTCTCCGG
1450	1460	1470	1480	1490	1500	1510	1520	1530
TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAAGGGAC	TCTGGTCAAG	GTCTCTGTAG	CTAGCACCAA	GGGCCCATCG	GTCTTCCCCC
ACCTGCTGCC	CCGGACCAAA	CGAATGACCC	CGGTTCCCTG	AGACCAGTGC	CAGAGACATC	GATCGTGGTT	CCCGGGTAGC	CAGAAAGGGG
1540	1550	1560	1570	1580	1590	1600	1610	1620
TGGCACCCCTC	CTCCAAGAGC	ACCTCTGGGG	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCGGTG	ACGGTGTCTG
ACCGTGGGAG	GAGGTTCTCG	TGGAGACCCC	CGTGTGCGCG	GGACCCGACG	GACCAGTTCC	TGATGAAGGG	GCTTGGCCAC	TGCCACAGCA
1630	1640	1650	1660	1670	1680	1690	1700	1710
GGAACCTCAGG	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCTTA	CAGTCTCTAG	GACTCTACTC	CCTCAGCAGC	GTGGTCAACG
CCTTGAGTCC	GCGGGACTGG	TCGCCGCACG	TGTGGAAGGG	CCGACAGGAT	GTCAAGGATC	CTGAGATGAG	GGAGTCGTGC	CACCAGTGGC
1720	1730	1740	1750	1760	1770	1780	1790	1800
TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCT	ACATCTGCAA	CGTGAATCAC	AAGCCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTTGGTGAGA
ACGGAGGATC	GTGGAACCCG	TGGGTCTGGA	TGTAGACGTT	GCACCTTAGT	TTCGGGTCTG	TGTGGTTCCA	CCTGTTCTTT	CAACCACTCT

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FIG. 14C

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1810	1820	1830	1840	1850	1860	1870	1880	1890
GGCCAGCACA	GGGAGGAGG	GTGTCTGCTG	GAAGCAGGC	TCAGCGCTCC	TGCGTGACG	CATCCGGCT	ATGCAGCCC	AGTCCAGGC
CCGTCGTGT	CCCTCCCTCC	CACAGACGAC	CTTCGGTCCG	AGTCGCGAGG	ACGACCTGC	GTAGGCGGA	TACGTGCGG	TCAGGTCCCG
1900	1910	1920	1930	1940	1950	1960	1970	1980
AGCAAGGCAG	GCCCCGTCTG	CCCTCTTACC	CGAGGCCTC	TGCCCGCCCC	ACTCATGTC	AGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG
TGCTTCCGTC	CGGGGCAGAC	GGAGAACTGG	GCCTCCGGAG	ACGGGCGGG	TGAGCTG	TCCCTCTCC	AGAAGACCGA	AAAAGGGGTC
1990	2000	2010	2020	2030	2040	2050	2060	2070
GCTCTGGCA	GGCACAGGCT	AGGTGCCCC	AACCCAGGC	CTGCACACAA	AGGGGCGAGT	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC
CGAGACCCGT	CCGTGTCCGA	TCCACGGGGA	TTGGGTCCGG	GACGTGTGTT	TCCCCGTCCA	CGACCCGAGT	CTGGACGGTT	CTCGGTATAG
2080	2090	2100	2110	2120	2130	2140	2150	2160
CGGGAGGACC	CTGCCCTTGA	CCTAAGCCCC	CCCCAAGGC	CAAACTCTCC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA
GCCCTCCTGG	GACGGGGACT	GGATTCTGGT	GGGTTTCCG	GTTTGAGAGG	TGAGGGAGTC	GAGCCTGTGG	AAGAGAGGAG	GGTCTAAGGT
2170	2180	2190	2200	2210	2220	2230	2240	2250
GTAATCCCA	ATCTTCTCTC	TGCAGAGCCC	AAATCTTGTG	ACAAACTCA	CACATGCCCA	CCGTGCCCCAG	GTAAGCCAGC	CCAGGCCCTCG
CATTGAGGT	TAGAAGAGAG	ACGTCTCGGG	TTTAGAACAC	TGTTTGTAGT	GTGTACGGG	TGCACGGGTC	CATTGCGTGC	GGTCCGGAGC
2260	2270	2280	2290	2300	2310	2320	2330	2340
CCCTCCAGCT	CAAGGCGGGA	CAGGTGCCCT	AGAGTAGCCT	GCATCCAGGG	ACACACCACG	TGGGTACCAA	CATGTCCGGA	GCCACATGGA
GGGAGGTCGA	GTTCCGCCCT	GTCCACGGGA	TCTCATCGGA	CGTAGGTCCC	TGTGTGGTGC	ACCCATGGTT	GTACAGGCCCT	CGGTGTACCT
2350	2360	2370	2380	2390	2400	2410	2420	2430
CAGAGGCCGG	CTCGGCCAC	CCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	CCTCTGTCCC	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA
GTCTCCGGCC	GAGCCGGGTG	GGAGACGGGA	CTCTCACTGG	CGACATGGTT	GGAGACAGGG	ATGTCCCCTC	GGGGCTCTTG	GTGTCCACAT
2440	2450	2460	2470	2480	2490	2500	2510	2520
CACCCTGCCC	CCATCCCGGG	ATGAGCTGAC	CAAGAACCAAG	GTCAGCCTGA	CCTGCCCTGGT	CAAAGGCTTC	TATCCCAGCG	ACATCGCCGT
GTGGGACGGG	GGTAGGGCCC	TACTCGACTG	GTTCTTGCTC	CAGTCGGACT	GGACGGACCA	GTTTCCGAG	ATAGGGTCGC	TGTAGCGGCA
2530	2540	2550	2560	2570	2580	2590	2600	2610
GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACNAG	ACCACGCCTC	CCGTGCTGGA	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA
CCTCACCCCTC	TCGTTACCCG	TCGGCCCTCT	GTTGATGTTT	TGGTGGGGAG	GGCACCACCT	GAGGCTGCCG	AGGAGAGAGG	AGATGTCGTT
2620	2630	2640	2650	2660	2670	2680	2690	2700
GCTCACCGTG	GACAAGAGCA	GGTGGCAGCA	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	ACACGCGAGAA
CGAGTGGCAC	CTGTTCTCGT	CCACCGTCGT	CCCCCTGCG	AAGAGTACGA	GGCACTACGT	ACTCCGAGAC	GTGTTGGTGA	TGTGCGTCTT

FIG. 14D

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2710	2720	2730	2740	2750	2760	2770	2780	2790
GAGCCTCTCC	CTGTCTCCGG	GTAATGAGT	GGACGGCCG	GCAAGCCCC	GCTCCCCGG	CTCTCGGGT	CGCACGAGGA	TGCTTGGCAC
CTCGGAGAGG	GACAGAGGCC	CATTACTCA	CGCTGCCGGC	CGTTCCGGGG	CGAGGGGCC	GAGAGCGCCA	GCCTGCTCCT	ACGAACCCGTG
2800	2810	2820	2830	2840	2850	2860	2870	2880
GTACCCCTCG	TACATACTTC	CCGGGGCCCC	AGCATGGAAA	TAAAGCACCC	AGCGCTGCC	TGGGCCCTTG	CGAGACTGTG	ATGGTTCTTT
CATGGGGAC	ATGTATGAG	GGCCCGCGGG	TCGTACCTTT	ATTTCGTGG	TCGCGACGG	ACCCGGGGAC	GCTCTGACAC	TACCAAGAAA
2890	2900	2910	2920	2930	2940	2950	2960	2970
CCACGGGTCA	GGCCGAGTCT	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	GGGTCCCACT	GTCCCCACAC	TGGCCCCAGGC	TGTGCAGGTG
GGTGCCCACT	CCGGCTCAGA	CTCCGGACTC	ACCGTACTCC	CTCCGTCTCG	CCCAGGGTGA	CAGGGGTGTG	ACCGGGTCCG	ACACGTCCAC
2980	2990	3000	3010	3020	3030	3040	3050	3060
TGCCCTGGCC	CCCTAGGGTG	GGGCTCAGCC	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	AGCAGCACCT
ACGGACCCCG	GGGATCCAC	CCCGAGTCGG	TCCCCGACGG	GAGCCGTCCC	ACCCCTAAA	CGGTGCGACC	GGGAGGGAGG	TCGTGCTGGA
3070	3080	3090	3100	3110	3120	3130	3140	3150
GCCCTGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCTGGG	GACAGACACA	CAGCCCTGC	CTCTGTAGGA	GACTGTCTTG	TTCTGTGAGC
CGGGACCCGA	CCCGGTGCC	TTTCGGGATCC	TCGGGGACCC	CTGTCTGTGT	GTCGGGGACG	GAGACATCCT	CTGACAGGAC	AAGACACTCG
3160	3170	3180	3190	3200	3210	3220	3230	3240
GCCCTGTCC	TCCCGACTTC	CATGCCCACT	CGGGGGCATG	CCTAGTCCAT	GTGGGTAGGG	ACAGGCCCTC	CCTCACCCAT	CTACCCCCAC
CGGGGACAGG	AGGGCTGGAG	GTACGGGTGA	GCCCCCGTAC	GGATCAGGTA	CACGCATCCC	TGTCCGGGAG	GGAGTGGGTA	GATGGGGGTG
3250	3260	3270	3280	3290	3300	3310	3320	3330
GGCACTAACC	CCTGGCTGCC	CTGCCCCAGCC	TCGCACCCGC	ATGGGGACAC	AACCGACTCC	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG
CCGTGATTGG	GGACCCGACGG	GACGGGTCCG	AGCGTGGGCG	TACCCCTGTG	TTGGCTGAGG	CCCCGTGACG	TGAGAGCCCCG	GGACACCTCC
3340	3350	3360	3370	3380	3390	3400	3410	3420
GACTGGTGCA	GATGCCACACA	CACACACTCA	GCCCAGACCC	GTTCAACAAA	CCCCGCACTG	AGGTTGGCCG	GCCACACGGC	CACCACACAC
CTGACCACGT	CTACGGGTGT	GTGTGTGAGT	CGGGTCTGGG	CAAGTTGTTT	GGGGCGTGAC	TCCAACCGGC	CGGTGTGCCG	GTGGTGTGTG
3430	3440	3450	3460	3470	3480	3490	3500	3510
ACACGTGCAC	GCCTACACACA	CGGAGCCTCA	CCCGGGCGAA	CTGCACAGCA	CCCAGACCAG	AGCAAGGTCC	TGCGACACGT	GAACACTCCT
TGTGCACGTG	CGGAGTGTGT	GCCTCGGAGT	GGGCCCGCTT	GACGTGTCGT	GGGTCTGGTC	TCGTTCCAGG	AGCGTGTGCA	CTTGTGAGGA
3520	3530	3540	3550	3560	3570	3580	3590	3600
CGGACACAGG	CCCCACGAG	CCCCACGGCG	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	TCAGACAAAC
GCCTGTGTCC	GGGGTGTCTC	GGGGTGGGCC	GTGGAGTTCC	GGGTGCTCGG	AGAGCCGTCC	AAGAGGTGTA	CGACTGGACG	AGTCTGTTTG

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FIG. 14E

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3610 CCAGCCCTCC TCTCACAGG GTGCCCCTGC AGCGCCACA CACACACAGG GGATCACACA CCACGTACG TCCCTGGCCC TGGCCACTT 3690
 GGTCCGGAGG AGAGTGTTC CACGGGGACG TCGGCGGTGT GTGTGTGTCC CCTAGTGTG GGTGCAGTGC AGGACCGGG ACCGGGTGAA 3680
 3700 CCCAGTGCCG CCCTTCCCTG CAGGACGGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCTTCC CCGTGCCCTT 3780
 GGGTCACGGC GGGAAAGGAC GTCTGCCTA GTCGGAGCTG ACACGGAAGA TCAACGGTCG GTAGACAACA AACGGGGAGG GGGACGGAA 3770
 3790 CCTTGACCTT GGAAGGTGCC ACTCCACTG TCCTTTCCTA ATAAATGAG GAAATTGCAT CGCATTTGCT GAGTAGGTGT CATTCTATT 3870
 GGAACCTGGG CCTTCCACG TGAGGGTGAC AGGAAAGGAT TATTTTACTC CTTTAACGTA GCGTAACAGA CTCATCCACA GTAAGATAAG 3860
 3880 TGGGGGTGG GGTGGGGCAG GACAGCAAGG GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT ATGGCTTCTG 3960
 ACCCCCCACC CCACCCCGTC CTGTCTGTTCC CCTCCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTAGC CCACCCGAGA TACCGAAGAC 3950
 3970 AGGCGGAAAG AACCAGCTGG GGCTCTAGG GGTATCCCCA CGCGCCCTGT AGCGGCGCAT TAAGCGCGG 4030 4040 4050
 TCCGCTTTC TTGGTCGACC CCGAGATCCC CCATAGGGGT GCAGGGGACA TCGCCCGTA ATTCGGCGG CCACACACC CAATGCGCGT 4000
 4060 GCGTGACCG TACACTGCC AGCGCCTAG AGCGCCTAG GGTATCCCCA CGCGCCCTGT AGCGGCGCAT TAAGCGCGG 4120 4130 4140
 CGCACTGGC ATGTGAACG TCGCGGATC TCGCGGATC GCGCGGAGG AAGCGGAGG AAGAGCGGTG CAAGCGGCGG GGTGCGCGG CCTCTCAAAA 4110
 4150 AAGGGAATAA AAGCATGCAT CTCATTAGT CAGCAACCAT AGTCCCGCCC CTAACCTCCG CCATCCCGCC CCATTAACCTCG CCCAGTTCCG 4230
 TTCCCTTTT TTCGTACGTA GAGTTAATCA GTCGTTGGTA TCAGGGCGGG GATTGAGCG GGTAGGGCGG GGTAGGAGC GGTCAAGGC 4220 4200 4210 4220 4320
 4240 CCCATTCTCC GCCCATGGC TGACTAATTT TTTTATTTA TGCAGAGGCC GAGGCGGCTT CCGCCTCTGA GCTATTCCAG AAGTAGTGAG 4310 4300 4310 4320
 GGGTAAGAG CGGGGTACCG ACTGATTAA ACTGATTAA AAAATAAAT ACCTCTCCG CTCCGGCGGA GCCGGAGACT CGATAAGTC TTCATCACTC 4310 4300 4310 4320
 4330 GAGGCTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTTG ACAGCTCAGG GCTGCGATT CCGGCCAAT CCGGCCAAT TTTGACGGCA TCCTAGCGTG 4410
 CTCGGAATA ACCTCCGGAT CCGAATAACGT TTTTCGAAC TGTCGAGTCC CGACGCTAAA GCGCGGTTG AACTGCCGT AGGATCGCAC 4400 4410 4400 4410
 4420 AAGGCTGGTA GGATTTTATC CCGCTGCCA TCATGTTTCG ACCATTGAAC TGCATCGTC CCGTGTCCTA CCGTGTCCTA AAATATGGG ATTGGCAAGA 4490 4480 4490 4500
 TTCCGACCAT CCTAAATAG GGGGACGGT AGTACCAAGC TGGTAACCTG ACGTAGCAGC GGCACAGGGT TTTATACCTC TAACCGTTCT 4480 4490 4500 4510

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FIG. 14F

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4510	4520	4530	4540	4550	4560	4570	4580	4590
ACGAGACCT	ACCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	AGAATGACCA	CAACCTCTTC	AGTGGAAAGT	AAACAGAATC
TGCCTCTGGA	TGGGACCGGA	GCGAGTCCT	TGCTCAAGTT	CATGAAGGTT	TCTTACTGGT	GTTGGAGAAG	TCACCTTCCA	TTTGTCTTAG
4600	4610	4620	4630	4640	4650	4660	4670	4680
TGGTGATTAT	GGGTAGGAAA	ACCTGGTTCT	CCATTCCTGA	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	AGTAGAGAAC
ACCACTAATA	CCCATCCTTT	TGGACCAAGA	GGTAAGGACT	CTTCTTAGCT	GGAATTTCC	TGTCCTTAAT	ATATCAAGAG	TCACTCTCTG
4690	4700	4710	4720	4730	4740	4750	4760	4770
TCAAGAACCC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG	TTTGGATGAT	GCCTTAAGAC	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG
AGTTTCTTGG	TGGTGCTCCT	CGAGTAAAG	AACGGTTTTC	AAACCTACTA	CGGAATTCTG	AATAACTTGT	TGGCCTTAAC	CGTTCATTTC
4780	4790	4800	4810	4820	4830	4840	4850	4860
TAGACATGGT	TTGGATAGTC	GGAGGCAGTT	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	ACAAGGATCA
ATCTGTACCA	AACCTATCAG	CCTCCGTCAA	GACAAATGGT	CCTTCGGTAC	TTAGTTGGTC	CGGTGGAATC	TGAGAAACAC	TGTTCTCTAGT
4870	4880	4890	4900	4910	4920	4930	4940	4950
TGCAGGAATT	TGAAAGTGAC	ACGTTTTTCC	CAGAAATTGA	TTTGGGNA	TATAAATTC	TCCCAGAATA	CCCAGGCGTC	CTCTCTGAGG
ACGTCCTTAA	ACTTTTCACTG	TGCAAAAAGG	GTCTTTAACT	AAACCCCTTT	ATATTGAAG	AGGTCCTTAT	GGTCCGCGAG	GAGAGACTCC
4960	4970	4980	4990	5000	5010	5020	5030	5040
TCCAGGAGGA	AAAAGGCATC	AAGTATAAGT	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	GCTCCCCCTCC
AGGTCCTCCT	TTTTCCGTAG	TTCATATTCA	AACCTCAGAT	GCTCTTCTTT	CTGATTGTCC	TTCTACGAAA	GTTCAAGAGA	CGAGGGGAGG
5050	5060	5070	5080	5090	5100	5110	5120	5130
TAAAGCTATG	CATTTTATA	AGACCATGGG	ACTTTTGCTG	GCTTTAGATC	TCCTTGTGAA	GGAACCTTAC	TTCTGTGGTG	TGACATAATT
ATTTTCGATAC	GTAAAAATAT	TCTGGTACCC	TGAAAAACGAC	CGAAATCTAG	AGAAACACTT	CCTTGGAAATG	AAGACACCAC	ACTGTATTAA
5140	5150	5160	5170	5180	5190	5200	5210	5220
GGACAAACTA	CCTACAGAGA	TTTAAAGCTC	TAAAGTAAAT	ATAAAATTTT	TAAGTGATA	ATGTGTTAAA	CTACTGATTTC	TAATGTGTTG
CCTGTTTGAT	GGATGTCTCT	AAATTTCGAG	ATTCCATTTA	TATTTTAAAA	ATTCACATAT	TACACAATTT	GATGACTAAG	ATTAACAAAC
5230	5240	5250	5260	5270	5280	5290	5300	5310
TGTATTTTAG	ATTCCAACCT	ATGGAACCTGA	TGAATGGGAG	CAGTGGTGA	ATGCCCTTAA	TGAGGAAAAC	CTGTTTGTCT	CAGAAGAAAT
ACATAAAATC	TAAAGTTGGA	TACCTTGACT	ACTTACCCTC	GTCACCACCT	TACGGAAAT	ACTCCTTTTG	GACAAAACGA	GTCTTCTTTA
5320	5330	5340	5350	5360	5370	5380	5390	5400
GCCATCTAGT	GATGATGAGG	CTACTGCTGA	CTCTCAACAT	TCTACTCCTC	CAAAAAAGAA	GAGAAAGGTA	GAAGACCCCA	AGGACTTCC
CGGTAGATCA	CTACTACTCC	GATGACGACT	GAGAGTTGTA	AGATGAGGAG	GTTTTCTTCT	CTCTTTCCAT	CTTCTGGGGT	TCCTGAAAGG

FIG. 14G

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5410 TTCAGAAATG CTAAGTTTTT 5420 TGAGTCATGC 5430 TGCTGTTAGT 5440 TGTGTTTAGT 5450 AATAGAACTC 5460 TTGCTTGCTT 5470 TGCTATTAC 5480 ACCACAAAGG 5490 AAAAAGCTGC
 AAGTCTTAAC GATTCAAAAAA 5420 ACTCAGTACG 5430 ACTCAGTACG 5440 ACACAAATCA 5450 TTATCTTGAG 5460 AACGAAACGAA 5470 ACGATAAAAG 5480 TGGTGTTTCC 5490 TTTTTCGACG
 5500 ACTGCTATAC AAGAAAATTA 5510 TGGAAAAATA 5520 TGGAAAAATA 5530 TTCTGTAACC 5540 TTTATAAGTA 5550 GGCATAACAG 5560 TTATAATCAT 5570 AACATACTGT 5580 TTTTTCCTTAC
 TGACGATATG TTCTTTTAAT 5510 ACCTTTTAT 5520 AAGACATGG 5530 AATATTCTAT 5540 CCGTATTGTC 5550 AATATTAGTA 5560 TTGTATGACA 5570 AAAAAGAAATG
 5590 TCCACACAGG CATAGAGTGT 5600 CTGCTATTAA 5610 TAACTATGCT 5620 CAAAAATTGT 5630 GTACCTTTAG 5640 CTTTTTAATT 5650 TGTAAAGGGG 5660 TTAATAAGGA 5670 TTAATAAGGA
 AAGTGTGTCC GTATCTCACA 5600 GACGATAAAT 5610 ATTGATACGA 5620 GTTTTAAACA 5630 CATGGAAATC 5640 GAAAAATTAA 5650 ACAATTTCCC 5660 AATTATTCTT
 5680 ATATTTGATG TATAGTGCCT 5690 TGACTAGAGA 5700 TCATAATCAG 5710 CCATACCACA 5720 TTTGTAGAGG 5730 TTTTACTTGC 5740 TTTAAAAAAC 5750 CTCCCACACC 5760 CTCCCACACC
 TATAAACTAC ATATCACGGA 5690 ACTGATCTCT 5700 AGTATTAGTC 5710 GGTATGGTGT 5720 AAACATCTCC 5730 AAAATGAACG 5740 AAAATTTTGG 5750 GAGGGTGTGG 5760 GAGGGTGTGG
 5770 TCCCCCTGAA CCTGAAACAT 5780 AAAATGAATG 5790 CAATTGTTGT 5800 TGTTAACTTG 5810 TTTATTGCAG 5820 CTTATAATGG 5830 TTACAAATAA 5840 AGCAATAGCA 5850 AGCAATAGCA
 AGGGGGACTT GGACTTTGTA 5780 TTTTACTTAC 5790 GTTAAACAACA 5800 ACAATTGAAC 5810 GAATATTACC 5820 GAATATTACC 5830 AATGTTTATT 5840 TCCTTATCTG 5850 TCCTTATCTG
 5860 TCACAAATTT CACAAATAAA 5870 GCATTTTTTTT 5880 CACTGCATTC 5890 TAGTTGTGGT 5900 TTGTCCAAAC 5910 TCATCAATGT 5920 ATCTTATCAT 5930 GTCTGGATCG 5940 GTCTGGATCG
 AGTGTTTAAA GTGTTTATTT 5870 CGTAAAAAAA 5880 CCGCTAGAGT 5890 ACACCTCAA 5900 GAAGCGGGTG 5910 GGGTTGAACA 5920 AATAACGTCG 5930 AATATTACCA 5940 ATGTTTATTT
 5950 GCTGGATGAT CCTCCAGCGC 5960 GGGGATCTCA 5970 TGCTGGAGTT 5980 CTTGCCCCAC 5990 CCCAACTTGT 6000 CCAAACTTGT 6010 TTATTGCAGC 6020 TTATAATGGT 6030 TACAAATAAA
 CGACCTACTA GGAGGTCGCG 5960 CCGCTAGAGT 5970 CCGCTAGAGT 5980 ACACCTCAA 5990 GAAGCGGGTG 6000 GGGTTGAACA 6010 AATAACGTCG 6020 AATATTACCA 6030 ATGTTTATTT
 6040 GCAATAGCAT CACAAATTC 6050 ACAAATAAAG 6060 CATTTTTTTC 6070 ACTGCATTCT 6080 AGTTGTGGTT 6090 TGTCCAAACT 6100 CATCAATGTA 6110 TCTTATCATG 6120 TCTTATCATG
 CGTTATCGTA GTGTTTAAAG 6050 TGTTTATTTT 6060 GTAAAAAAG 6070 GTAAAAAAG 6080 TGACGTAAGA 6090 TCAACACCAA 6100 ACAGGTTTGA 6110 GTAGTTACAT 6120 AGAATAGTAC
 6130 TCTGTATACC GTCGACCTCT 6140 AGCTAGAGCT 6150 TGGCGTAATC 6160 ATGGTCATAG 6170 CTGTTTCCCTG 6180 TGTGAAATGG 6190 TTATCCGCTC 6200 ACAATTCAC 6210 ACAATTCAC
 AGACATATGG CAGCTGGAGA 6140 TCGATCTCGA 6150 ACCGCATTAG 6160 TACCATGATC 6170 GACAAAGGAC 6180 ACACTTTAAC 6190 AATAGGCGAG 6200 TGTTAAGGTG 6210 TGTTAAGGTG
 6220 ACAACATACG AGCCGGAAGC 6230 ATAAAGTGTA 6240 AAGCCTGGGG 6250 TGCCTAATGA 6260 GTGAGCTAAC 6270 TCACATTAAT 6280 TGGGTTGCGC 6290 TCACCTGCCC 6300 TCACCTGCCC
 TGTGTATGC TCGGCCCTCG 6230 TATTTACAT 6240 TTCGGACCCC 6250 ACCGATTACT 6260 CACTCGATTG 6270 AGTGAATTA 6280 ACGCAACGCG 6290 AGTGACGGGC 6300 AGTGACGGGC

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FIG. 14H

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6310	6320	6330	6340	6350	6360	6370	6380	6390
CTTTCAGTC	GGGAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCCG
GAAAGGTCAG	CCCTTTGGAC	AGCACGGTCG	ACGTAATTAC	TTAGCCGGTT	GCGCGCCCCCT	CTCCGCCCAA	CGCATAAACC	GCGAGAAAGGC
6400	6410	6420	6430	6440	6450	6460	6470	6480
CTTCTCGCT	CACCTGACTCG	CTGCGCTCGG	TCGTTCCGGCT	GCGCGAGCG	GTATCAGCTC	ACTCAAAGSC	GGTAATACGG	TTATCCACAG
GAAGGAGCGA	GTGACTGAGC	GACGCGAGCC	AGCAAGCCGA	CGCCGCTCGC	CATAGTCGAG	TGAGTTTCCG	CCATTATGCC	AATAGGTGTC
6490	6500	6510	6520	6530	6540	6550	6560	6570
AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTCC
TTAGTCCCCCT	ATTGCGTCCT	TTCTTGATACA	CTCGTTTTTCC	GGTCGTTTTTC	CGGTCCCTTGG	CATTTTTTCCG	GCGCAACGAC	CGCAAAAAGG
6580	6590	6600	6610	6620	6630	6640	6650	6660
ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	AAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT
TATCCGAGGC	GGGGGACTG	CTCGTAGTGT	TTTTAGCTGC	GAGTTCAGTC	TCCACCGCTT	TGGGCTGTCC	TGATATTTCT	ATGGTCCGCA
6670	6680	6690	6700	6710	6720	6730	6740	6750
TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCCCTT	TCCTCCCTTCG	GGAAGCGTGG
AAGGGGGACC	TTCGAGGGAG	CACGCGAGAG	GACAAGGCTG	GGACGGCGAA	TGGCCTATGG	ACAGGCGGAA	AGAGGGAAGC	CCTTCGCACC
6760	6770	6780	6790	6800	6810	6820	6830	6840
CGCTTTCTCA	ATGCTCACGC	TGTAGGTATC	TCAGTTCGGT	GTAGTTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTTCAGC
GCGAAAGAGT	TACGAGTGGG	ACATCCATAG	AGTCAAGCCA	CATCCAGCAA	GCGAGGTTTCG	ACCCGACACA	CGTGCTTGGG	GGGCAAGTCG
6850	6860	6870	6880	6890	6900	6910	6920	6930
CCGACCGCTG	CGCCTTATCC	GGTAACATATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA
GGCTGGCGAC	GCGGAATAGG	CCATTGATAG	CAGAACTCAG	GTTGGGCCAT	TCTGTGCTGA	ATAGCGGTGA	CCGTGCTCGG	TGACCATTGT
6940	6950	6960	6970	6980	6990	7000	7010	7020
GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTGGTA
CCTAATCGTC	TCGCTCCATA	CATCCGCCAC	GATGCTCTCA	GAACTTCACC	ACCGGATTGA	TGCCGATGTG	ATCTTCTCTGT	CATAAACCAT
7030	7040	7050	7060	7070	7080	7090	7100	7110
TCCTGGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT
AGACGCGGAG	CGACTTCGGT	CAATGGAAGC	CTTTTTCTCA	ACCATCGAGA	ACTAGGCCGT	TTGTTTGGTG	GCGACCATCG	CCACCAAAAA
7120	7130	7140	7150	7160	7170	7180	7190	7200
TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	AAAAGGATC	TCAAGNAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAACG
AACAAACGTT	CGTCGTCTAA	TGCGCGTCTT	TTTTTCTCTAG	AGTCTCTCTA	GGAACCTAGA	AAAGATGCC	CAGACTGCGA	GTCACCTTGC

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FIG. 14I

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7210	7220	7230	7240	7250	7260	7270	7280	7290
AAACTCAG	TTAAGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	ACCTAGATCC	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA
TTTTTGAGTGC	AATTCCTAA	AACCAGTACT	CTAATAGTTT	TTCTCTAGAAG	TGGATCTAGG	AAAATTTAAT	TTTTACTTCA	AAATTTAGTT
7300	7310	7320	7330	7340	7350	7360	7370	7380
TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTTCAT
AGATTTTCATA	TATACTCAT	TGAACCCAGAC	TGTCATAGGT	TACGAATTAG	TCACTCCGTG	GATAGAGTCG	CTAGACAGAT	AAAGCAAGTA
7390	7400	7410	7420	7430	7440	7450	7460	7470
CCATAGTTGC	CTGACTCCCC	GTCGTGTAGA	TAACTACGAT	ACGGAGGGC	TTACCATCTG	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC
GGTATCAACG	GACTGAGGG	CAGCACATCT	ATTGATGCTA	TGCCCTCCCG	AATGGTAGAC	CGGGGTACAG	ACGTTACTAT	GGCGCTCTGG
7480	7490	7500	7510	7520	7530	7540	7550	7560
CACGCTCAC	GGTCCAGAT	TTATCAGCAA	TAAACCCAGCC	AGCCGGAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	TCCGCCCTCCA
GTGCGAGTGG	CCGAGGTCTA	AATAGTCGTT	ATTTGGTCGG	TCCGCCCTTCC	CGGCTCGCGT	CTTCACCAGG	ACGTTGAAAT	AGGCGGAGGT
7570	7580	7590	7600	7610	7620	7630	7640	7650
TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCCGCCAGTT	AATAGTTTGC	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG
AGGTCAGATA	ATTAACAACG	GCCCTTCGAT	CTCATTTATC	AAGCGGTCAA	TTATCAAACG	CGTTGCAACA	ACGGTAACGA	TGTCCGTAGC
7660	7670	7680	7690	7700	7710	7720	7730	7740
TGGTGTACG	CTCGTCGTTT	GGTATGGCTT	CATTACGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTGTGCAAAA
ACCACAGTGC	GAGCAGCAA	CCATACCGAA	GTAAGTCGAG	GCCAAAGGTT	GCTAGTTCCG	CTCAATGTAC	TAGGGGGTAC	AACACGTTTT
7750	7760	7770	7780	7790	7800	7810	7820	7830
AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTCAGAAG	TAAGTTGGCC	GCAGTGTAT	CACTCATGGT	TATGGCAGCA	CTGCATAATT
TTTCGCCAATC	GAGGAAGCCA	GGAGGCTAGC	AACAGTCTTC	ATTCAACCCG	CGTCACAATA	GTGAGTACCA	ATACCGTCGT	GACGTATTAA
7840	7850	7860	7870	7880	7890	7900	7910	7920
CTCTTACTGT	CATGCCATCC	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGCGACCGA
GAGAAATGACA	GTACGGTAGG	CATTCTACGA	AAAGACACTG	ACCACCTATG	AGTTGGTTCA	GTAAGACTCT	TATCACATAC	GCCGCTGGCT
7930	7940	7950	7960	7970	7980	7990	8000	8010
GTTGCTCTTG	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	ACTTTAAAG	TGCTCATCAT	TGGAACACGT	TCTTCGGGGC
CAACGAGAAC	GGGCGGCAGT	TATGCCCTAT	TATGGCGCGG	TGTATCGTCT	TGAATTTTC	ACGAGTAGTA	ACCTTTTGCA	AGAAGCCCCG
8020	8030	8040	8050	8060	8070	8080	8090	8100
GAAACTCTC	AAGGATCTTA	CCGCTGTGTA	GATCCAGTTC	GATGTAACCC	ACTCGTGCAC	CCAACTGATC	TTCAGCATCT	TTTACTTTCA
CTTTTGAGAG	TTCCTAGAAAT	GGCGACAACT	CTAGGTCAAG	CTACATTGGG	TGAGCACGTG	GGTTGACTAG	AAGTCGTAGA	AAATGAAAGT

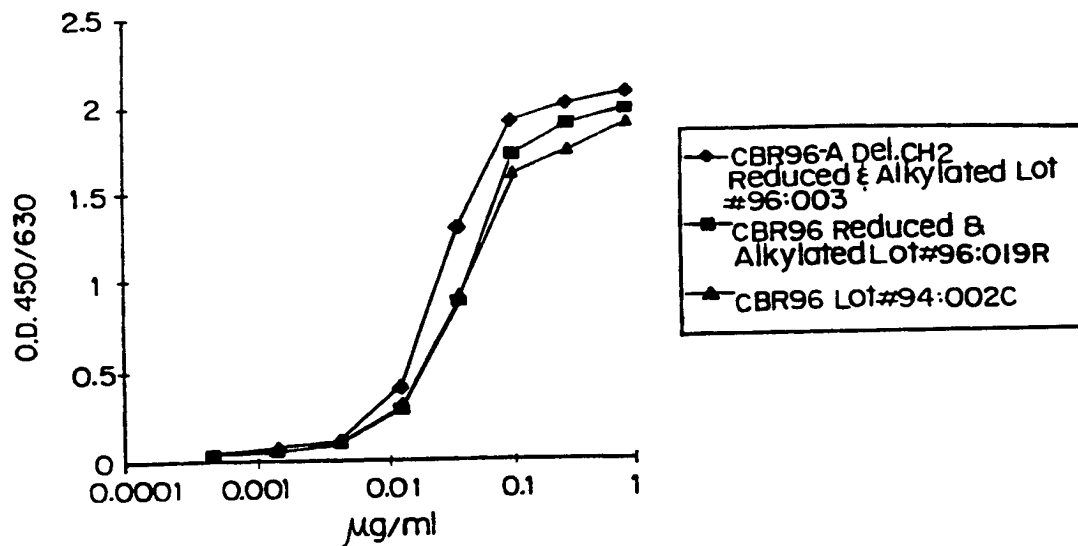
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FIG. 14J

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8110	8120	8130	8140	8150	8160	8170	8180	8190
CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAG	GGATAAGGG	CGACACGGAA	ATGTTGAATA	CTCATACTCT
GGTCGCAAG	ACCCACTCGT	TTTGTCCCT	CCGTTTACG	GCGTTTTTC	CCTTATCCC	GCTGTGCCCT	TACAACTTAT	GAGTATGAGA
8200	8210	8220	8230	8240	8250	8260	8270	8280
TCCTTTTTC	ATATTATTGA	AGCATTATC	AGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG
AGGAAAAAGT	TATAATAACT	TCGTAAATAG	TCCCAATAAC	AGAGTACTCG	CCTATGTATA	AACTTACATA	AATCTTTTAA	TTTGTTTATC
8290	8300	8310	8320	8330				
GGGTCCGCG	CACATTCCC	CGAAAAAGTG	CACCTGACGT	C				
CCCAAGGCG	GTGTAAGGG	GCTTTTCACG	GTGGACTGCA	G				

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Fig. 15COMPARISON OF WHOLE chiBR96 AND
DELETED CH2 chiBR96 ON Ley/K ELISA

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hBR96-2B:L235 to A235 and G237 to A237

hBR96-2C:E318 to S318, K320 to S320, and K322 to S322

hBR96-2D:P331 to A331

hBR96-2E:L235 to A235, G237 to A237, E318 to S318, K320 to S320,
and K322 to S322

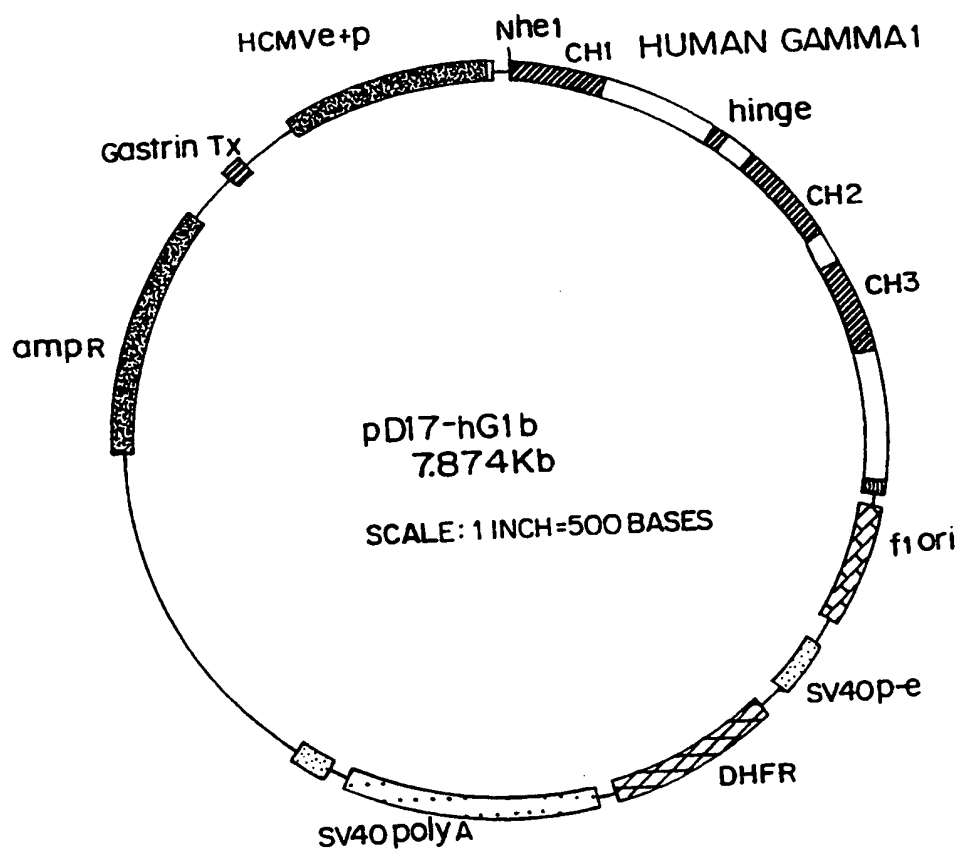
hBR96-2F:L235 to A235, G237 to A237, and P331 to A331

hBR96-2G:E318 to S318, K320 to S320, K322 to S322, and P331 to
A331

hBR96-2H: L235 to A235, G237 to A237, E318 to S318, K320 to S320,
K322 to S322, and P331 to A331

FIG. 16

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Fig. 17

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FIG. 18A

1	GGTACCAATT	TAAATTGATA	TCTCCTTAGG	TCTCGAGTCT	CTAGATAACC
51	GGTCAATCGA	TTGGAATTCT	TGCGGCCGCT	TGCTAGCCAC	CATGGAGTTG
101	TGGTTAAGCT	TGGTCTTCCT	TGTCCTTGTT	TTAAAAGGTG	TCCAGTGTGA
151	AGTGCAACTG	GTGGAGTCTG	GGGGAGGCTT	AGTGCAGCCT	GGAGGGTCCC
201	TGCGACTTTC	CTGTGCTGCA	TCTGGATTCC	CGTTCAGTGA	CTATTACATG
251	TATTGGGGTTC	GCCAGGCTCC	AGGCAAGGGA	CTGGAGTGGG	TCTCATACAT
301	TAGTCAAGAT	GGTGATATAA	CCGACTATGC	AGACTCCGTA	AAGGGTCGAT
351	TCACCATCTC	CAGAGACAAT	GCAAAGAACA	GCCTGTACCT	GCAAATGAAC
401	AGCCTGAGGG	ACGAGGACAC	AGCCGTGTAT	TACTGTGCAA	GAGGCCTGGC
451	GGACGGGGCC	TGGTTTGCTT	ACTGGGGCCA	AGGGACTCTG	GTCACGGTCT
501	CTTCCGCTAG	CACCAAGGGC	CCATCGGTCT	TCCCCCTGGC	ACCCTCCTCC
551	AAGAGCACCT	CTGGGGGCAC	AGCGGCCCTG	GGCTGCCTGG	TCAAGGACTA
601	CTTCCCCGAA	CCGGTGACGG	TGTCGTGGAA	CTCAGGCGCC	CTGACCAGCG
651	GCGTGACAC	CTTCCCGGCT	GTCCTACAGT	CCTCAGGACT	CTACTCCCTC
701	AGCAGCGTGG	TCACCGTGCC	CTCCAGCAGC	TGGGGCACCC	AGACCTACAT
751	CTGCAACGTG	AATCACAAGC	CCAGCAACAC	CAAGGTGGAC	AAGAAAGTTG
801	GTGAGAGGCC	AGCACAGGGA	GGGAGGGTGT	CTGCTGGAAG	CCAGGCTCAG
851	CGCTCCTGCC	TGGACGCATC	CCGGCTATGC	AGCCCCAGTC	CAGGGCAGCA
901	AGGCAGGCCC	CGTCTGCCTC	TTCACCCGGA	GGCCTCTGCC	CGCCCCACTC
951	ATGCTCAGGG	AGAGGGTCTT	CTGGCTTTTT	CCCCAGGCTC	TGGGCAGGCA
1001	CAGGCTAGGT	GCCCCTAACC	CAGGCCCTGC	ACACAAAGGG	GCAGGTGCTG
1051	GGCTCAGACC	TGCCAAGAGC	CATATCCGGG	AGGACCCTGC	CCCTGACCTA
1101	AGCCCACCCC	AAAGGCCAAA	CTCTCCACTC	CCTCAGCTCG	GACACCTTCT
1151	CTCCTCCCAG	ATTCCAGTAA	CTCCCAATCT	TCTCTCTGCA	GAGCCCCAAT
1201	CTTGTGACAA	AACTCACACA	TGCCCACCGT	GCCCAGGTAA	GCCAGCCCAG
1251	GCCTCGCCCT	CCAGCTCAAG	GCGGGACAGG	TGCCCTAGAG	TAGCCTGCAT
1301	CCAGGGACAG	GCCCCAGCCG	GGTGCTGACA	CGTCCACCTC	CATCTCTTCC

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1351	TCAGCACCTG	235 AACTCCTGGG	237 GGGACCGTCA	GTCTTCCTCT	TCCCCCAAA
1401	ACCCAAGGAC	ACCCTCATGA	TCTCCCGGAC	CCCTGAGGTC	ACATGCGTGG
1451	TGGTGGACGT	GAGCCACGAA	GACCCTGAGG	TCAAGTTCAA	CTGGTACGTG
1501	GACGGCGTGG	AGGTGCATAA	TGCCAAGACA	AAGCCGCGGG	AGGAGCAGTA
1551	CAACAGCACG	TACCGTGTGG	TCAGCGTCCT	CACCGTCCTG	CACCAGGACT
1601	GGCTGAATGG	318 CAAGGAGTAC	320 AAGTGCAAGG	322 TCTCCAACAA	AGCCCTCCCA
1651	331 GCCCCATCG	AGAAAACCAT	CTCCAAAGCC	AAAGGTGGGA	CCCGTGGGGT
1701	GCGAGGGCCA	CATGGACAGA	GGCCGGCTCG	GCCCACCCTC	TGCCCTGAGA
1751	GTGACCGCTG	TACCAACCTC	TGTCCCTACA	GGGCAGCCCC	GAGAACCACA
1801	GGTGATACAC	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG	AACCAGGTCA
1851	GCCTGACCTG	CCTGGTCAAA	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG
1901	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT
1951	GCTGGACTCC	GACGGCTCCT	TCTTCCTCTA	CAGCAAGCTC	ACCGTGGACA
2001	AGAGCAGGTG	GCAGCAGGGG	AACGTCTTCT	CATGCTCCGT	GATGCATGAG
2051	GCTCTGCACA	ACCACTACAC	GCAGAAGAGC	CTCTCCCTGT	CTCCGGGTAA
2101	ATGAGTGCGA	CGGCCGGCAA	GCCCCGCTC	CCCGGGCTCT	CGCGGTGCGA
2151	CGAGGATGCT	TGGCACGTAC	CCCCTGTACA	TACTTCCCGG	GCGCCCAGCA
2201	TGGAAATAAA	GCACCCAGCG	CTGCCCTGGG	CCCCTGCGAG	ACTGTGATGG
2251	TTCTTTCCAC	GGGTCAGGCC	GAGTCTGAGG	CCTGAGTGGC	ATGAGGGAGG
2301	CAGAGCGGGT	CCCCTGTGCC	CCACACTGGC	CCAGGCTGTG	CAGGTGTGCC
2351	TGGGCCCCCT	AGGGTGGGGC	TCAGCCAGGG	GCTGCCCTCG	GCAGGGTGGG
2401	GGATTTGCCA	GCGTGGCCCT	CCCTCCAGCA	GCACCTGCCC	TGGGCTGGGC
2451	CACGGGAAGC	CCTAGGAGCC	CCTGGGGACA	GACACACAGC	CCCTGCCTCT
2501	GTAGGAGACT	GTCCTGTTCT	GTGAGCGCCC	CTGTCCTCCC	GACCTCCATG
2551	CCCCTCGGG	GGCATGCCTA	GTCCATGTGC	GTAGGGACAG	GCCCTCCCTC
2601	ACCCATCTAC	CCCCACGGCA	CTAACCCCTG	GCTGCCCTGC	CCAGCCTCGC
2651	ACCCGCATGG	GGACACAACC	GA CTCCGGGG	ACATGCACTC	TCGGGCCCCTG
2701	TGGAGGGACT	GGTGCAGATG	CCCACACACA	CACTCAGCCC	AGACCCGTTT
2751	AACAAACCCC	GCACTGAGGT	TGGCCGGCCA	CACGGCCACC	ACACACACAC
2801	GTGCACGCCT	CACACACGGA	GCCTCACCCG	GGCGAACTGC	ACAGCACCCA

FIG. 18B

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2851	GACCAGAGCA	AGGTCCTCGC	ACACGTGAAC	ACTCCTCGGA	CACAGGCCCC
2901	CACGAGCCCC	ACGCGGCACC	TCAAGGCCCA	CGAGCCTCTC	GGCAGCTTCT
2951	CCACATGCTG	ACCTGCTCAG	ACAAACCCAG	CCCTCCTCTC	ACAAGGGTGC
3001	CCCTGCAGCC	GCCACACACA	CACAGGGGAT	CACACACCAC	GTCACGTCCC
3051	TGGCCCTGGC	CCACTTCCCA	GTGCCGCCCT	TCCCTGCAGG	ACGGATCAGC
3101	CTCGACTGTG	CCTTCTAGTT	GCCAGCCATC	TGTTGTTTGC	CCCTCCCCCG
3151	TGCCTTCCTT	GACCCTGGAA	GGTGCCACTC	CCACTGTCCT	TTCCTAATAA
3201	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCATT	CTATTCTGGG
3251	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA	GACAATAGCA
3301	GGCATGCTGG	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAACC
3351	AGCTGGGGCT	CTAGGGGGTA	TCCCCACGCG	CCCTGTAGCG	GCGCATTAAAG
3401	CGCGGCGGGT	GTGGTGGTTA	CGCGCAGCGT	GACCGCTACA	CTTGCCAGCG
3451	CCCTAGCGCC	CGCTCCTTTC	GCTTTCCTTC	CTTCCTTCT	CGCCACGTTT
3501	GCCGGGCCTC	TCAAAAAGG	GAAAAAAGC	ATGCATCTCA	ATTAGTCAGC
3551	AACCATAGTC	CCGCCCCATA	CTCCGCCCCAT	CCCGCCCCATA	ACTCCGCCCCA
3601	GTTCCGCCCA	TTCTCCGCCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA
3651	GAGGCCGAGG	CCGCCTCGGC	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG
3701	CTTTTTTGGG	GGCCTAGGCT	TTTGCAAAA	GCTTGGACAG	CTCAGGGCTG
3751	CGATTTTCGG	CCAAACTTGA	CGGCAATCCT	AGCGTGAAGG	CTGGTAGGAT
3801	TTTATCCCCG	CTGCCATCAT	GGTTCGACCA	TTGAACTGCA	TCGTCGCCGT
3851	GTCCCAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC
3901	TCAGGAACGA	GTTCAAGTAC	TTCCAAAGAA	TGACCACAAC	CTCTTCAGTG
3951	GAAGGTAAAC	AGAATCTGGT	GATTATGGGT	AGGAAAACCT	GGTTCTCCAT
4001	TCCTGAGAAG	AATCGACCTT	TAAAGGACAG	AATTAATATA	GTTCTCAGTA
4051	GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	ATTTTCTTGC	CAAAAGTTTG
4101	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	GTAAAGTAGA
4151	CATGGTTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAGGAA	GCCATGAATC
4201	AACCAGGCCA	CCTTAGACTC	TTGTGACAA	GGATCATGCA	GGAATTTGAA
4251	AGTGACACGT	TTTTCCAGAA	AATTGATTG	GGGAAATATA	AACTTCTCCC
4301	AGAATACCCA	GGCGTCCTCT	CTGAGGTCCA	GGAGGAAAAA	GGCATCAAGT

FIG. 18C

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4351	ATAAGTTTGA	AGTCTACGAG	AAGAAAGACT	AACAGGAAGA	TGCTTTCAAG
4401	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	CATGGGACTT
4451	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC
4501	ATAATTGGAC	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAAATATAA
4551	AATTTTAAAG	TGTATAATGT	GTAAACTAC	TGATTCTAAT	TGTTTGTGTA
4601	TTT TAGATTC	CAACCTATGG	AACTGATGAA	TGGGAGCAGT	GGTGGAATGC
4651	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	AGAAATGCCA	TCTAGTGATG
4701	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	AAAGAAGAGA
4751	AAGGTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCTAA	GTTTTTTGAG
4801	TCATGCTGTG	TTTAGTAATA	GAACTCTTGC	TTGCTTTGCT	ATTTACACCA
4851	CAAAGGAAAA	AGCTGCACTG	CTATACAAGA	AAATTATGGA	AAAATATTCT
4901	GTAACCTTTA	TAAGTAGGCA	TAACAGTTAT	AATCATAACA	TACTGTTTTT
4951	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	TATTAATAAC	TATGCTCAAA
5001	AATTGTGTAC	CTTTAGCTTT	TTAATTTGTA	AAGGGGTAA	TAAGGAATAT
5051	TTGATGTATA	GTGCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTTG
5101	TAGAGGTTTT	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG
5151	AAACATAAAA	TGAATGCAAT	TGTTGTTGTT	AACTTGTTTA	TTGCAGCTTA
5201	TAATGGTTAC	AAATAAAGCA	ATAGCATCAC	AAATTTCAAC	AATAAAGCAT
5251	TTTTTTTCACT	GCATTCTAGT	TGTGGTTTGT	CCAAACTCAT	CAATGTATCT
5301	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	ATCTCATGCT
5351	GGAGTTCTTC	GCCCACCCCA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA
5401	AATAAAGCAA	TAGCATCACA	AATTTCACAA	ATAAAGCATT	TTTTTCACTG
5451	CATTCTAGTT	GTGGTTTGTC	CAAACTCATC	AATGTATCTT	ATCATGTCTG
5501	TATACCGTCG	ACCTCTAGCT	AGAGCTTGGC	GTAATCATGG	TCATAGCTGT
5551	TTCTGTGTG	AAATTGTTAT	CCGCTCACAA	TTCCACACAA	CATACGAGCC
5601	GGAAGCATAA	AGTGTAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAACTCAC
5651	ATTAATTGCG	TTGCGCTCAC	TGCCCCGCTT	CCAGTCGGGA	AACCTGTCGT
5701	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTTCGT
5751	ATTGGGCGCT	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	GCTCGGTCGT
5801	TCGGCTGCGG	CGAGCGGTAT	CAGCTCACTC	AAAGGCGGTA	ATACGGTTAT

FIG. 18D

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5851	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	AAAAGGCCAG
5901	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	TTTTCCATAG
5951	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT
6001	GGCGAAACCC	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC
6051	TCCCTCGTGC	GCTCTCCTGT	TCCGACCCTG	CCGCTTACCG	GATACCTGTC
6101	CGCCTTTCTC	CCTTCGGGAA	GCGTGCGCT	TTCTCAATGC	TCACGCTGTA
6151	GGTATCTCAG	TTCGGTGTAG	GTCGTTGCT	CCAAGCTGGG	CTGTGTGCAC
6201	GAACCCCCCG	TTCAGCCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT
6251	TGAGTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG
6301	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG
6351	AAGTGGTGGC	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG
6401	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT
6451	CCGSCAAACA	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	TTGCAAGCAG
6501	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC
6551	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG
6601	TCATGAGATT	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATTAAAAA
6651	TGAAGTTTTA	AATCAATCTA	AAGTATATAT	GAGTAAACTT	GGTCTGACAG
6701	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	CTCAGCGATC	TGTCTATTTC
6751	GTTTCATCCAT	AGTTGCCTGA	CTCCCCGTCG	TGTAGATAAC	TACGATACGG
6801	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA	ATGATACCGC	GAGACCCACG
6851	CTCACC GGCT	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGAAGGGCCG
6901	AGCGCAGAAG	TGGTCCTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAAT
6951	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTCG	CCAGTTAATA	GTTTGCGCAA
7001	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT	GTCACGCTCG	TCGTTTG GTA
7051	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	TACATGATCC
7101	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC	TTCGGTCCTC	CGATCGTTGT
7151	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC
7201	ATAATTCTCT	TACTGTCATG	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT
7251	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG
7301	CTCTTGCCCC	GCGTCAATAC	GGGATAATAC	CGCGCCACAT	AGCAGAACTT

FIG. 18E

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7351	TAAAAGTGCT	CATCATTGGA	AAACGTTCTT	CGGGGCGAAA	ACTCTCAAGG
7401	ATCTTACCGC	TGTTGAGATC	CAGTTCGATG	TAACCCACTC	GTGCACCCAA
7451	CTGATCTTCA	GCATCTTTTA	CTTTCACCAG	CGTTTCTGGG	TGAGCAAAAA
7501	CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT
7551	TGAATACTCA	TACTCTTCCT	TTTTCAATAT	TATTGAAGCA	TTTATCAGGG
7601	TTATTGTCTC	ATGAGCGGAT	ACATATTTGA	ATGTATTTAG	AAAAATAAAC
7651	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	AAGTGCCACC	TGACGTCGAC
7701	GGATCGGGAG	ATCTGCTAGG	TGACCTGAGG	CGCGCCGGCT	TCGAATAGCC
7751	AGAGTAACCT	TTTTTTTTAA	TTTTATTTTA	TTTTATTTTT	GAGATGGAGT
7801	TTGGCGCCGA	TCTCCCGATC	CCCTATGGTC	GACTCTCAGT	ACAATCTGCT
7851	CTGATGCCGC	ATAGTTAAGC	CAGTATCTGC	TCCCTGCTTG	TGTGTTGGAG
7901	GTCGCTGAGT	AGTGCGCGAG	CAAAATTTAA	GCTACAACAA	GGCAAGGCTT
7951	GACCGACAAT	TGCATGAAGA	ATCTGCTTAG	GGTTAGGCGT	TTTGCCTGTC
8001	TTGCGGATGT	ACGGGCCAGA	TATACGCGTT	GACATTGATT	ATTGACTAGT
8051	TATTAATAGT	AATCAATTAC	GGGGTCATTA	GTTCATAGCC	CATATATGGA
8101	GTTCCGCGTT	ACATAACTTA	CGGTAAATGG	CCCGCCTGGC	TGACCGCCCA
8151	ACGACCCCCG	CCCATTGACG	TCAATAATGA	CGTATGTTCC	CATAGTAACG
8201	CCAATAGGGA	CTTTCCATTG	ACGTCAATGG	GTGGACTATT	TACGGTAAAC
8251	TGCCCCACTTG	GCAGTACATC	AAGTGTATCA	TATGCCAAGT	ACGCCCCCTA
8301	TTGACGTCAA	TGACGGTAAA	TGGCCCGCCT	GGCATTATGC	CCAGTACATG
8351	ACCTTATGGG	ACTTTCCTAC	TTGGCAGTAC	ATCTACGTAT	TAGTCATCGC
8401	TATTACCATG	GTGATGCGGT	TTTGGCAGTA	CATCAATGGG	CGTGGATAGC
8451	GGTTTGACTC	ACGGGGATTT	CCAAGTCTCC	ACCCCATTTGA	CGTCAATGGG
8501	AGTTTGTTTT	GGCACCAAAA	TCAACGGGAC	TTTCCAAAAT	GTCGTAACAA
8551	CTCCGCCCCA	TTGACGCAAA	TGGGCGGTAG	GCGTGTACGG	TGGGAGGTCT
8601	ATATAAGCAG	AGCTCTCTGG	CTAACTAGAG	AACCCACTGC	TTACTGGCTT
8651	ATCGAAATTA	ATACGACTCA	CTATAGGGAG	ACCCAAGCTT	

FIG. 18F

SUBSTITUTE SHEET (RULE 26)

pD17-hG1b

SUBSTITUTE SHEET (RULE 26)

pD17-hG1b

322	1210	1220	1230331	1240	1250	1260
	GCAAGGTC	CAACAAAGCC	CTCCAGCCC	CCATCGAGAA	AACCATCTCC	AAAGCCAAG
	CGTTCCAGAG	GTTGTTTCGG	GAGGTCGGG	GGTAGCTCTT	TTGGTAGAGG	TTTCGGTTTC
1270	1280	1290	1300	1310	1320	
	GTGGGACCG	TGGGGTGCGA	GGGCCACATG	GACAGAGGCC	GGCTCGGCCC	ACCCTCTGCC
	CACCCCTGGC	ACCCACGCT	CCCGTGTAC	CTGTCTCCGG	CCGAGCCGGG	TGGGAGACGG
1330	1340	1350	1360	1370	1380	
	CTGAGAGTGA	CCGCTGTACC	AACCTCTGTC	CCTACAGGGC	AGCCCGAGA	ACCACAGGTG
	GACTCTCACT	GGCGACATGG	TTGGAGACAG	GGATGTCCCG	TCGGGGCTCT	TGGTGTCCAC
1390	1400	1410	1420	1430	1440	
	TACACCCCTGC	CCCCATCCCC	GGATGAGCTG	ACCAAGAACC	AGGTCAGCCT	GACCTGCCCTG
	ATGTGGGACG	GGGGTAGGGC	CCTACTCGAC	TGGTTCTTGG	TCCAGTCCGA	CTGGACGGAC
1450	1460	1470	1480	1490	1500	
	GTCAAAGGCT	TCTATCCCAG	CGACATCGCC	GTGGAGTGGG	AGAGCAATGG	GCAGCCGGAG
	CAGTTTCCGA	AGATAGGGTC	GCTGTAGCGG	CACCTCACCC	TCTCGTTACC	CGTCGGCCCTC
1510	1520	1530	1540	1550	1560	
	AACAATAACA	AGACCACGCC	TCCCGTGCTG	GACTCCGACG	GCTCCTTCTT	CCTCTACAGC
	TTGTTGATGT	TCTGGTGCGG	AGGGCACGAC	CTGAGGCTGC	CGAGGAAGAA	GGAGATGTGC
1570	1580	1590	1600	1610	1620	
	AAGTCAACCG	TGGACAAGAG	CAGGTGGCAG	CAGGGGAACG	TCTTCTCATG	CTCCGTGATG
	TTCCAGTGGC	ACCTGTTCTC	GTCCACCCTGC	GTCCCCCTGC	AGAAGAGTAC	GAGGCACTAC
1630	1640	1650	1660	1670	1680	
	CATGAGGCTC	TGCACAACCA	CTACACGCAG	AAGAGCCTCT	CCCTGTCTCC	GGGTAAATGA
	GTACTCCGAG	ACGTGTTGGT	GATGTGGCTC	TTCTCGGAGA	GGGACAGAGG	CCCATTACT
1690	1700	1710	1720	1730	1740	
	GTGCGACGGC	CGGCAAGCCC	CCGCTCCCGG	GGCTCTCGCG	GTGCGACGAG	GATGCTTGGC
	CACGCTGCCG	GCCGTTCCGG	GGCGAGGGGC	CCGAGAGCGC	CAGCGTGCTC	CTACGAACCG
1750	1760	1770	1780	1790	1800	
	ACGTACCCCC	TGTACATACT	TCCCGGGCGC	CCAGCATGGA	AATAAGCAC	CCAGCGGTGC
	TGCATGGGG	ACATGTATGA	AGGGCCCCGC	GGTCGTACCT	TTATTTCTGT	GGTCGCGACG

FIG. 19D

FIG. 19D		pD17-hG1b				
1810	1820	1830	1840	1850	1860	
CCCTGGGCCC	TGCGAGACTG	TGATGGTTCT	TTCCACGGGT	CAGGCCGAGT	CTGAGGCCCTG	
GGACCCGGGG	ACGCTCTGAC	ACTACCAAGA	AAGTGCCCA	GTCCGGCTCA	GACTCCGGAC	
1870	1880	1890	1900	1910	1920	
AGTGGCATGA	GGGAGCAGAA	GCGGGTCCCA	CTGTCCCCAC	ACTGGCCCAG	GCTGTGCAGG	
TCACCGTACT	CCCTCCGTCT	CGCCAGGGT	GACAGGGGTG	TGACCGGGTC	CGACACGTCC	
1930	1940	1950	1960	1970	1980	
TGTGCTGGG	CCCCCTAGGG	TGGGGCTCAG	CCAGGGGCTG	CCCTCGGCAG	GGTGGGGGAT	
ACACGGACCC	GGGGGATCCC	ACCCCGAGTC	GGTCCCCGAC	GGGAGCCGTC	CCACCCCCCTA	
1990	2000	2010	2020	2030	2040	
TTGCCAGCGT	GGCCCTCCCT	CCAGCAGCAC	CTGCCCTGGG	CTGGGCCACG	GGAGAGCCCTA	
AACGGTCGCA	CCGGGAGGGA	GGTCGTCGTG	GACGGGACCC	GACCCGGTGC	CCTTCGGGAT	
2050	2060	2070	2080	2090	2100	
GGAGCCCCCTG	GGGACAGACA	CACAGCCCCCT	GCCTCTGTAG	GAGACTGTCC	TGTTCTGTGA	
CCTCGGGGAC	CCCTGTCTGT	GTGTGCGGGA	CGGAGACATC	CTCTGACAGG	ACAAGACACT	
2110	2120	2130	2140	2150	2160	
GCGCCCTGT	CCTCCGACC	TCCATGCCCA	CTCGGGGCA	TGCTGGGGAT	GCGGTGGGCT	
CGCGGGGACA	GGAGGGCTGG	AGGTACGGGT	GAGCCCCCGT	ACGAGCCCTA	CGCCACCCGA	
2170	2180	2190	2200	2210	2220	
CTATGGCTTC	TGAGCGGAA	AGAACCACT	GGGGCTCTAG	GGGGTATCCC	CACCGGCCT	
GATACCGAAG	ACTCCGCTT	TCTTGTGCGA	CCCCGAGATC	CCCCATAGGG	GTGCGCGGGA	
2230	2240	2250	2260	2270	2280	
GTAGCGGCG	ATTAGCGCG	GCGGGTGTGG	TGGTTACGGG	CAGCGTGACC	GCTACACTTG	
CATCGCGCG	TAATTGCGC	CGCCCAAC	ACCAATGCGC	GTGCGACTGG	CGATGTGAAC	
2290	2300	2310	2320	2330	2340	
CCAGGCGCT	AGCGCCGCT	CCTTTCGCTT	TCTTCCCTTC	CTTTCGCG	ACGTTCCGCG	
GGTCGCGGGA	TCGCGGGCGA	GGAAAGCGAA	AGAAAGGGAAG	GAAAGAGCGG	TGCAAGCGCG	
2350	2360	2370	2380	2390	2400	
GCTTCCCCG	TCAAGCTCTA	AATCGGGCA	TCCCTTTAGG	GTTCGGAATT	AGTGCCTTAC	
CGAAAGGGGC	AGTTGAGAT	TTAGCCCCGT	AGGAAATCC	CAAGGCTAAA	TCACGAATG	

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FIG. 19E		pD17-hG1b	
2410	2420	2430	2440
GGCACCTCGA	CCCCAAAAA	CTTGATTAGG	GTGATGTTTC
CCGTGGAGCT	GGGGTTTTT	GAACTAATCC	CACTACCAAG
2470	2480	2490	2500
GATAGACGGT	TTTTGCCCC	TTGACGTTGG	AGTCCACGTT
CTATCTGCCA	AAAAGCGGGA	AACTGCAACC	TCAGGTGCAA
2530	2540	2550	2560
TCCAAACTGG	AACAACACTC	AACCCTATCT	CGGTCTATTTC
AGGTTTGACC	TTGTTGTGAG	TTGGGATAGA	GCCAGATAAG
2590	2600	2610	2620
TGGGATTTC	GGCCTATTGG	TTAAAAAATG	AGCTGATTTA
ACCCCTAAAG	CCGGATAACC	AATTTTTTAC	TCGACTAAAT
2650	2660	2670	2680
AATTCGTGG	AATGTGTGTC	AGTTAGGGTG	TGGAAAGTCC
TTAAGACACC	TTACACACAG	TCAATCCAC	ACCTTTCAGG
2710	2720	2730	2740
GAAGTATGCA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT
CTTCATACGT	TTCTGTACGTA	GAGTTAATCA	GTCTTTGGTA
2770	2780	2790	2800
CCATCCCGCC	CCTAACTCCG	CCCAGTTCCG	CCCATTTCTC
GGTAGGGCCG	GGATTGAGGC	GGGTCAAGGC	GGGTAAAGAG
2830	2840	2850	2860
TTTTTATTTA	TGCAGAGGCC	GAGGCCGCCCT	CGGCCTCTGA
AAAAATAAAT	ACGTCTCCGG	CTCCGGCGGA	GCCGGAGACT
2890	2900	2910	2920
GAGGCTTTTT	TGGAGGCCCTA	GGCTTTTGCA	AAAAGCTTGG
CTCCGAAAAA	ACCTCCGGAT	CCGAAAACGT	TTTTTCGAACC
2950	2960	2970	2980
CGCGCCAAAC	TTGACGGCNA	TCCTAGCGTG	AAGGCTGGTA
GGCGGGTTTG	AACTGCCGTT	AGGATCGCAC	TTCCGACCAT
			3000
			CCCGCTGCCA
			GGGCGACGGT

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FIG. 19F		pD17-hG1b	
3010	3020	3030	3040
TCATGGTTCCG	ACCATTGAAC	TGCATCGTCG	CCGTGTCCCA
AGTACCAAGC	TGGTAACTTG	ACGTAGCAGC	GGCACAGGGT
3070	3080	3090	3100
ACGGAGACCT	ACCTTGGCCT	CCGCTCAGGA	ACGAGTTCAA
TGCCTCTGGA	TGGGACCCGA	GGCGAGTCCT	TGCTCAAGTT
3130	3140	3150	3160
CAACTCTTTC	AGTGGNAGGT	AAACAGNATC	TGGTGATTAT
GTTGGAGAAG	TCACCTTCCA	TTTGTCTTAG	ACCACATAATA
3190	3200	3210	3220
CCATTCCTGA	GAAGAATCGA	CCTTTAAAGG	ACAGAAATTAA
GGTAAGGACT	CTTCTTAGCT	GGAAATTTCC	TGTCCTTAATT
3250	3260	3270	3280
TCAAGAACC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG
AGTTTCITGG	TGGTGCTCCT	CGAGTAAAG	AACGGTTTTC
3310	3320	3330	3340
TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT
AATAACTTGT	TGGCCTTAAC	CGTTCATTTT	ATCTGTACCA
3370	3380	3390	3400
CTGTTTACCA	GGAGGCCATG	AATCAACCAG	GCCACCTTAG
GACAAATGGT	CCTTCCGTAC	TTAGTTGGTC	CGGTGGAATC
3430	3440	3450	3460
TGCAGGAATT	TGAAGTGAC	ACGTTTTTCC	CAGAAATTGA
ACGTCCCTTA	ACTTTCACCTG	TGCAAAAAGG	GTCTTTAACT
3490	3500	3510	3520
TCCCAGAATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA
AGGGTCTTAT	GGGTCCGCAG	GAGAGACTCC	AGGTCTCTCT
3550	3560	3570	3580
TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT
AACCTCAGAT	GCTCTTCTTT	CTGATTGTCC	TTCTACGAAA
			3590
			CAAGTTCTCT
			GTTCAAGAGA
			3600
			GCTCCCTCC
			CGAGGGGAGG

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FIG. 19G		pD17-hG1b	
3610	TAAAGCTATG	3650	GCTTTAGATC
3620	CATTTTATA	3660	TCTTTGTGAA
3630	GTAAAAATAT		AGAAACACTT
3640		3650	CGAAATCTAG
3650	AGACCATGGG	3660	TCTTTGTGAA
3660	TCTGGTACCC		AGAAACACTT
3670		3670	CCTACAGAGA
3680	TTCTGTGGTG	3720	TTTAAAGCTC
3690	AAGACACCAC		AAATTTTCGAG
3700		3710	GGATGTCCTC
3710	TGACATAATT	3720	TTTAAAGCTC
3720	ACTGTATTAA		AAATTTTCGAG
3730		3730	CTACTGATTC
3740	TAAGTGTATA	3780	TAATTTGTTG
3750	ATCCCAATTT		ATTAACAAAC
3760	TATTTTAAAA	3770	GATGACTAAG
3770		3780	CTACTGATTC
3780	TAAGTGTATA	3790	TAATTTGTTG
3790	ATCCCAATTT		ATTAACAAAC
3800	TATTTTAAAA	3800	CTACTGATTC
3810		3810	TAATTTGTTG
3820	TAAGTGTATA		ATTAACAAAC
3830	ATCCCAATTT	3830	CAGTGGTGGA
3840	TATTTTAAAA		ATGCCTTTAA
3850		3840	TACGAAATTT
3860	TAAGTGTATA		TACGAAATTT
3870	ATCCCAATTT	3850	GATGACTAAG
3880	TATTTTAAAA		CTACTGATTC
3890		3860	CTACTGATTC
3900	TAAGTGTATA		CTACTGATTC
3910	ATCCCAATTT	3870	GATGACTAAG
3920	TATTTTAAAA		CTACTGATTC
3930		3880	GATGACTAAG
3940	TAAGTGTATA		CTACTGATTC
3950	ATCCCAATTT	3890	GATGACTAAG
3960	TATTTTAAAA		CTACTGATTC
3970		3900	CTACTGATTC
3980	TAAGTGTATA		CTACTGATTC
3990	ATCCCAATTT	3910	GATGACTAAG
4000	TATTTTAAAA		CTACTGATTC
4010		3920	GATGACTAAG
4020	TAAGTGTATA		CTACTGATTC
4030	ATCCCAATTT	3930	GATGACTAAG
4040	TATTTTAAAA		CTACTGATTC
4050		3940	GATGACTAAG
4060	TAAGTGTATA		CTACTGATTC
4070	ATCCCAATTT	3950	GATGACTAAG
4080	TATTTTAAAA		CTACTGATTC
4090		3960	GATGACTAAG
4100	TAAGTGTATA		CTACTGATTC
4110	ATCCCAATTT	3970	GATGACTAAG
4120	TATTTTAAAA		CTACTGATTC
4130		3980	GATGACTAAG
4140	TAAGTGTATA		CTACTGATTC
4150	ATCCCAATTT	3990	GATGACTAAG
4160	TATTTTAAAA		CTACTGATTC
4170		4000	GATGACTAAG
4180	TAAGTGTATA		CTACTGATTC
4190	ATCCCAATTT	4010	GATGACTAAG
4200	TATTTTAAAA		CTACTGATTC
4210		4020	GATGACTAAG
4220	TAAGTGTATA		CTACTGATTC
4230	ATCCCAATTT	4030	GATGACTAAG
4240	TATTTTAAAA		CTACTGATTC
4250		4040	GATGACTAAG
4260	TAAGTGTATA		CTACTGATTC
4270	ATCCCAATTT	4050	GATGACTAAG
4280	TATTTTAAAA		CTACTGATTC
4290		4060	GATGACTAAG
4300	TAAGTGTATA		CTACTGATTC
4310	ATCCCAATTT	4070	GATGACTAAG
4320	TATTTTAAAA		CTACTGATTC
4330		4080	GATGACTAAG
4340	TAAGTGTATA		CTACTGATTC
4350	ATCCCAATTT	4090	GATGACTAAG
4360	TATTTTAAAA		CTACTGATTC
4370		4100	GATGACTAAG
4380	TAAGTGTATA		CTACTGATTC
4390	ATCCCAATTT	4110	GATGACTAAG
4400	TATTTTAAAA		CTACTGATTC
4410		4120	GATGACTAAG
4420	TAAGTGTATA		CTACTGATTC
4430	ATCCCAATTT	4130	GATGACTAAG
4440	TATTTTAAAA		CTACTGATTC
4450		4140	GATGACTAAG
4460	TAAGTGTATA		CTACTGATTC
4470	ATCCCAATTT	4150	GATGACTAAG
4480	TATTTTAAAA		CTACTGATTC
4490		4160	GATGACTAAG
4500	TAAGTGTATA		CTACTGATTC
4510	ATCCCAATTT	4170	GATGACTAAG
4520	TATTTTAAAA		CTACTGATTC
4530		4180	GATGACTAAG
4540	TAAGTGTATA		CTACTGATTC
4550	ATCCCAATTT	4190	GATGACTAAG
4560	TATTTTAAAA		CTACTGATTC
4570		4200	GATGACTAAG
4580	TAAGTGTATA		CTACTGATTC
4590	ATCCCAATTT	4210	GATGACTAAG
4600	TATTTTAAAA		CTACTGATTC
4610		4220	GATGACTAAG
4620	TAAGTGTATA		CTACTGATTC
4630	ATCCCAATTT	4230	GATGACTAAG
4640	TATTTTAAAA		CTACTGATTC
4650		4240	GATGACTAAG
4660	TAAGTGTATA		CTACTGATTC
4670	ATCCCAATTT	4250	GATGACTAAG
4680	TATTTTAAAA		CTACTGATTC
4690		4260	GATGACTAAG
4700	TAAGTGTATA		CTACTGATTC
4710	ATCCCAATTT	4270	GATGACTAAG
4720	TATTTTAAAA		CTACTGATTC
4730		4280	GATGACTAAG
4740	TAAGTGTATA		CTACTGATTC
4750	ATCCCAATTT	4290	GATGACTAAG
4760	TATTTTAAAA		CTACTGATTC
4770		4300	GATGACTAAG
4780	TAAGTGTATA		CTACTGATTC
4790	ATCCCAATTT	4310	GATGACTAAG
4800	TATTTTAAAA		CTACTGATTC
4810		4320	GATGACTAAG
4820	TAAGTGTATA		CTACTGATTC
4830	ATCCCAATTT	4330	GATGACTAAG
4840	TATTTTAAAA		CTACTGATTC
4850		4340	GATGACTAAG
4860	TAAGTGTATA		CTACTGATTC
4870	ATCCCAATTT	4350	GATGACTAAG
4880	TATTTTAAAA		CTACTGATTC
4890		4360	GATGACTAAG
4900	TAAGTGTATA		CTACTGATTC
4910	ATCCCAATTT	4370	GATGACTAAG
4920	TATTTTAAAA		CTACTGATTC
4930		4380	GATGACTAAG
4940	TAAGTGTATA		CTACTGATTC
4950	ATCCCAATTT	4390	GATGACTAAG
4960	TATTTTAAAA		CTACTGATTC
4970		4400	GATGACTAAG
4980	TAAGTGTATA		CTACTGATTC
4990	ATCCCAATTT	4410	GATGACTAAG
5000	TATTTTAAAA		CTACTGATTC

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FIG. 19H

pD17-hG1b			
4210	4220	4230	4240
CTTTTAAAT	TGTAAGGGG	TTAATAAGGA	ATATTGATG
GAAAAATTAA	ACATTCCCC	AATTATTCCT	TATAAACTAC
			ATAJACGGGA
			ACTGATCTCT
4270	4280	4290	4300
TCATAATCAG	CCATACCACA	TTTGTAAGG	TTTTACTGC
AGTATTAGTC	GGTATGGTGT	AAACATCTCC	AAAATGAACG
			AAATTTTTTG
4330	4340	4350	4360
TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT
AGGGGGACTT	GGACTTTGTA	TTTTACTTAC	GTTAACACA
			ACAATTGAC
4390	4400	4410	4420
CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT
GAATATTACC	AATGTTTATT	TCGTTATCGT	AGTGTTTAAA
			GCATTTTTTT
4450	4460	4470	4480
CACTGCATTC	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT
GTGACGTAAG	ATCAACACCA	AACAGGTTTG	AGTAGTTACA
			TAGAATAGTA
4510	4520	4530	4540
GCTGGATGAT	CCTCCAGCGC	GGGATCTCA	TGCTGGAGTT
CGACCTACTA	GGAGGTCGGG	CCCCTAGAGT	ACGACCTCAA
			GAAGCGGGTG
4570	4580	4590	4600
TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT
AATAACGTCG	AATATTACCA	ATGTTTATTT	CGTTATCGTA
			CACAAATTC
4630	4640	4650	4660
CATTTTTTTC	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT
GTAATAAAG	TGACGTAAGA	TCAACACCAA	ACAGGTTTGA
			ATGGTCATAG
4690	4700	4710	4720
TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC
AGACATATGG	CAGCTGGAGA	TCGATCTCGA	ACCGCATTAG
			TACCAAGTATC
4750	4760	4770	4780
TGTGAAATTG	TTATCCGCTC	ACAAATCCAC	ACAACATACG
ACACTTTAAC	AATAGGCGAG	TGTTAAGGTG	TGTTGTATGC
			AGCCGGNAGC
			ATAAAGTGTA
			TATTTACAT

FIG. 19

FIG. 19J		pD17-hG1b			
5410	5420	5430	5440	5450	5460
CCGACCGCTG	CGCCTTATCC	GGTAACATATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT
GGCTGGCGAC	GCGGAATAGG	CCATTGATAG	CAGNACTCAG	GTGGGCCAT	TCTGTGCTGA
5470	5480	5490	5500	5510	5520
TATCGCCACT	GCGACGAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGCGGGTG
ATAGCGGTGA	CCGTGCTCGG	TGACCAATTGT	CCTAATCGTC	TGCTCCATA	CATCCGCCAC
5530	5540	5550	5560	5570	5580
CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTGTGTA
GATGTCTCAA	GAACCTTCACC	ACCGGATTGA	TGCCGATGTG	ATCTTCCTGT	CATAAACCAT
5590	5600	5610	5620	5630	5640
TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA
AGACGGGAGA	CGACTTCGGT	CAATGGAAGC	CTTTTCTCA	ACCATCGAGA	ACTAGGCCGT
5650	5660	5670	5680	5690	5700
AACAACCCAC	CGCTGGTAGC	GGTGGTTTTT	TGTTTGCAG	GCAGCAGATT	ACGCGCAGAA
TTGTTTGGTG	GCGACCATCG	CCACCAAAA	AACAAACGTT	CGTCGTCTAA	TGCGCGTCTT
5710	5720	5730	5740	5750	5760
AAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGNACG
TTTTTCTTAG	AGTTCTTCTA	GGAACCTAGA	AAAGATGCCC	CAGACTGCGA	GTCACTCTTG
5770	5780	5790	5800	5810	5820
AAAACTCAG	TTAAGGGATT	TGGTCAATGA	GATTATCAA	AAGGATCTTC	ACCTAGATCC
TTTTTGAGTGC	AAITCCCATA	AACCCAGTACT	CTAATAGTTT	TTCTCTAGAAG	TGATCTTAGG
5830	5840	5850	5860	5870	5880
TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG
AAAATTTAAT	TTTTACTTCA	AAATTTAGTT	AGATTTCATA	TATACTCAT	TGAACCCAGAC
5890	5900	5910	5920	5930	5940
ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTTCAT
TGTCAATGGT	TACGAATTAG	TCACCTCCGTG	GATAGAGTCG	CTAGACAGAT	AAAGCAAGTA
5950	5960	5970	5980	5990	6000
CCATAGTTGC	CTGACTCCCC	GTGCGTAGA	TAACTACGAT	ACGGGAGGGC	TTACCATCTG
GGTATCAACG	GACTGAGGGG	CAGCACATCT	ATTGATGCTA	TGCCCTCCCCG	AATGGTAGAC

FIG. 19X

6010	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	TTATCAGCNA
	CGGGGTACGG	ACGTTACTAT	GGCGCTCTGG	GTGCGAGTGG	CCGAGGTCTA	AATAGTCGTT
6070	TAAACGAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	TCCGCCTCCA
	AATTGGTCGG	TCGGCCCTTCC	CGGCTCGCGT	CTTCACCAGG	ACGTTGAAAT	AGGCGAGGT
6130	AGGTCAGATA	ATTAACAACG	GCCCTTCGAT	CTCATTTCATC	AAGCGGTCAA	TTATCAAAACG
	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTGCGCCAGTT	AATAGTTTGC
6190	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACAG	CTCGTCGTTT	GGTATGGCTT
	CGTTGCAACA	ACGGTAACGA	TGTCCGTAGC	ACCACAGTGC	GAGCAGCAAA	CCATACCGBA
6250	CATTGAGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTGTGCAAAA
	GTAAGTCGAG	GCCAAGGGTT	GCTAGTTCCG	CTCAATGTAC	TAGGGGTAC	AACACGTTTT
6310	AAGCGGTTAG	CTCCTTTCGGT	CCTCCGATCG	TTGTGAGAAG	TAAGTTGGCC	GCAGTGTAT
	TTGCGCAATC	GAGGAAGCCA	GGAGGCTAGC	AACAGTCTTC	ATTCAACCGG	CGTCACATA
6370	CACTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	GTAAGATGCT
	GTGAGTACCA	ATACCGTCTGT	GACGTATTAA	GAGAAATGACA	GTACGGTAGG	CATTCTACGA
6430	TTTCTGTGAC	TGGTGAATAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGCGACCGA
	AAAGACACTG	ACCACTCATG	AGTTGGTTCA	GTAAGACTCT	TATCACATAC	GCCGCTGGCT
6490	GTGCTCTTGG	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	ACTTTAAAG
	CAACGAGAAC	GGGCCGCAGT	TATGCCCTAT	TATGGCGCGG	TGTATCTGCT	TGAAATTTTC
6550	TGCTCATCAT	TGGAAACGCT	TCCTCGGGGC	GAAAACCTCTC	AAGGATCTTA	CCGCTGTTGA
	ACGAGTAGTA	ACCTTTTGCA	AGAAGCCCCG	CTTTTGAAG	TTCTTAGAAT	GGCGACAAC

FIG. 19L

		pD17-hG1b	
6610	GATCCAGTTC	6650	TTTACTTTCA
6610	CTAGGTCAAG	6650	AAATGAAAGT
6620	GATGTAACCC	6660	
6620	CTACATTGGG	6660	
6630	ACTCGTGCAC	6640	CCAAGTATC
6630	TGAGCACGGT	6640	GGTTGACTAG
6640	AAACAGGAA	6700	CGCAAAAG
6640	TTTGTGCTT	6700	GGTATAGG
6650	TGGGTGAGCA	6710	CGCAAAAG
6650	ACCCACTCGT	6710	GGTATAGG
6660	GGTCCGCAAG	6720	GGTATAGG
6670	CCAGCGTTTC	6720	GGTATAGG
6670	GGTCCGCAAG	6720	GGTATAGG
6680	ATGTTGAATA	6730	AGCATTTATC
6680	TACAACTTAT	6730	TCGTAAATAG
6690	TCATCATGAGC	6740	AGCATTTATC
6690	AGAGTACTCG	6740	AGCATTTATC
6700	AGGTTATTG	6750	AGCATTTATC
6700	TCCCAATTAAC	6750	AGCATTTATC
6710	GGTTCCGCG	6760	AGCATTTATC
6710	CCCAAGGCGC	6760	AGCATTTATC
6720	TAGGTGACCT	6770	AGCATTTATC
6720	ATCCACTGGA	6770	AGCATTTATC
6730	TTTATTTTAT	6780	AGCATTTATC
6730	AAATAAATA	6780	AGCATTTATC
6740	CAGTACAATC	6790	AGCATTTATC
6740	GTCAATGTTAG	6790	AGCATTTATC
6750	GGAGTTCGCT	6800	AGCATTTATC
6750	CCTCCAGCGA	6800	AGCATTTATC
6760	CAATTGCATG	6810	AGCATTTATC
6760	GTTAACGTAC	6810	AGCATTTATC
6770	TTCTTAGACG	6820	AGCATTTATC
6770	TTCTTAGACG	6820	AGCATTTATC
6780	TTCTTAGACG	6830	AGCATTTATC
6780	TTCTTAGACG	6830	AGCATTTATC
6790	TTCTTAGACG	6840	AGCATTTATC
6790	TTCTTAGACG	6840	AGCATTTATC
6800	TTCTTAGACG	6850	AGCATTTATC
6800	TTCTTAGACG	6850	AGCATTTATC
6810	TTCTTAGACG	6860	AGCATTTATC
6810	TTCTTAGACG	6860	AGCATTTATC
6820	TTCTTAGACG	6870	AGCATTTATC
6820	TTCTTAGACG	6870	AGCATTTATC
6830	TTCTTAGACG	6880	AGCATTTATC
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6850	TTCTTAGACG	6900	AGCATTTATC
6850	TTCTTAGACG	6900	AGCATTTATC
6860	TTCTTAGACG	6910	AGCATTTATC
6860	TTCTTAGACG	6910	AGCATTTATC
6870	TTCTTAGACG	6920	AGCATTTATC
6870	TTCTTAGACG	6920	AGCATTTATC
6880	TTCTTAGACG	6930	AGCATTTATC
6880	TTCTTAGACG	6930	AGCATTTATC
6890	TTCTTAGACG	6940	AGCATTTATC
6890	TTCTTAGACG	6940	AGCATTTATC
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6910	TTCTTAGACG	6960	AGCATTTATC
6920	TTCTTAGACG	6970	AGCATTTATC
6920	TTCTTAGACG	6970	AGCATTTATC
6930	TTCTTAGACG	6980	AGCATTTATC
6930	TTCTTAGACG	6980	AGCATTTATC
6940	TTCTTAGACG	6990	AGCATTTATC
6940	TTCTTAGACG	6990	AGCATTTATC
6950	TTCTTAGACG	7000	AGCATTTATC
6950	TTCTTAGACG	7000	AGCATTTATC
6960	TTCTTAGACG	7010	AGCATTTATC
6960	TTCTTAGACG	7010	AGCATTTATC
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6970	TTCTTAGACG	7020	AGCATTTATC
6980	TTCTTAGACG	7030	AGCATTTATC
6980	TTCTTAGACG	7030	AGCATTTATC
6990	TTCTTAGACG	7040	AGCATTTATC
6990	TTCTTAGACG	7040	AGCATTTATC
7000	TTCTTAGACG	7050	AGCATTTATC
7000	TTCTTAGACG	7050	AGCATTTATC
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7010	TTCTTAGACG	7060	AGCATTTATC
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7020	TTCTTAGACG	7070	AGCATTTATC
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7050	TTCTTAGACG	7100	AGCATTTATC
7050	TTCTTAGACG	7100	AGCATTTATC
7060	TTCTTAGACG	7110	AGCATTTATC
7060	TTCTTAGACG	7110	AGCATTTATC
7070	TTCTTAGACG	7120	AGCATTTATC
7070	TTCTTAGACG	7120	AGCATTTATC
7080	TTCTTAGACG	7130	AGCATTTATC
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7100	TTCTTAGACG	7150	AGCATTTATC
7110	TTCTTAGACG	7160	AGCATTTATC
7110	TTCTTAGACG	7160	AGCATTTATC
7120	TTCTTAGACG	7170	AGCATTTATC
7120	TTCTTAGACG	7170	AGCATTTATC
7130	TTCTTAGACG	7180	AGCATTTATC
7130	TTCTTAGACG	7180	AGCATTTATC
7140	TTCTTAGACG	7190	AGCATTTATC
7140	TTCTTAGACG	7190	AGCATTTATC
7150	TTCTTAGACG	7200	AGCATTTATC
7150	TTCTTAGACG	7200	AGCATTTATC

FIG. 19M

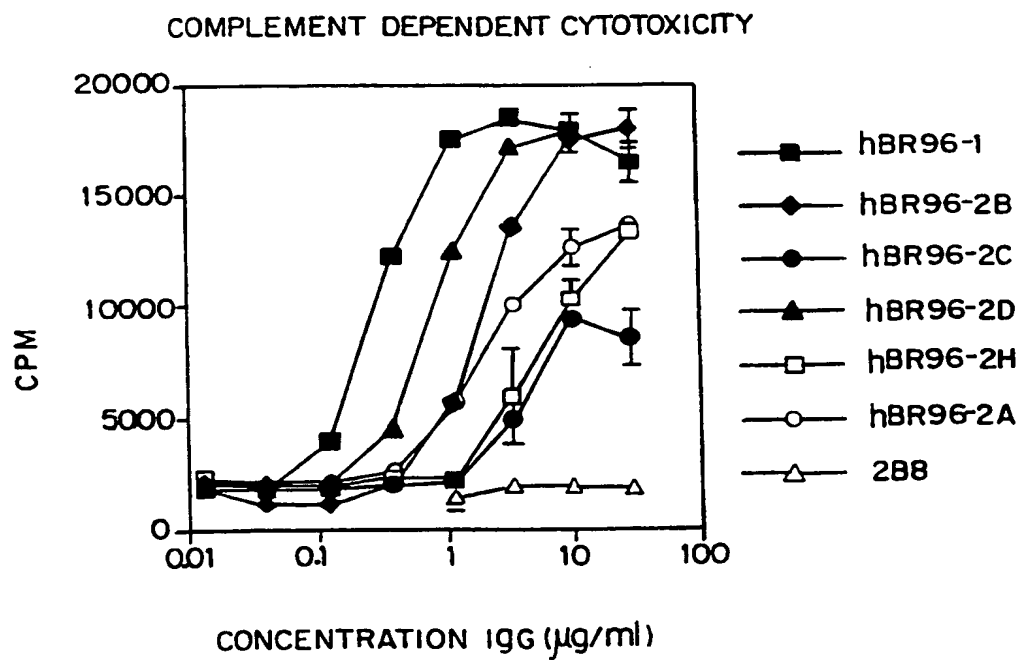
FIG. 19M		pD17-hG1b									
7210	CAGATATACG	7220	GATTATTGAC	7230	TAGTTATTAA	7240	TAGTAATCAA	7250	TTACGGGGTC	7260	TAATGCCCCAG
7210	GTCTATATGC	7220	GCAACTGTAA	7230	CTAATACTG	7240	ATCAATAATT	7250	ATCAATTAGT	7260	AAATGCCCCAG
7270	ATTAGTTTCAT	7280	AGCCCAATATA	7290	TGGAGTTCCG	7300	CGTTACATAA	7310	CTTACGGTAA	7320	ATGGCCCGCC
7270	TAATCAAGTA	7280	TCGGGTATAT	7290	ACCTCAAGGC	7300	GCAATGTAAT	7310	GAATGCCAAT	7320	TACCGGGCGG
7330	TGGCTGACCG	7340	CCCAACGACC	7350	CCCGCCCAAT	7360	GACGTCAATA	7370	ATGACGTATG	7380	TTCCCATAGT
7330	ACCGACTGGC	7340	GGGTTGCTGG	7350	GGCGGGGTAA	7360	CTGCAGTTAT	7370	TACTGCATAC	7380	AAGGGTATCA
7390	AACGCCAATA	7400	GGGACTTTCC	7410	ATTGACGTCA	7420	ATGGGTGGAC	7430	TATTTACGGT	7440	AAACTGCCCA
7390	TTGCGGTTAT	7400	CCCTGAAAGG	7410	TAACTGCAGT	7420	TACCCACCTG	7430	ATAAATGCCA	7440	TTTGACGGGT
7450	CTTGGCAGTA	7460	CATCAAGTGT	7470	ATCATATGCC	7480	AAGTACGCCC	7490	CCTATTGACG	7500	TCAATGACCG
7450	GAACCGTCTAT	7460	GTAGTTTACA	7470	TAGTATACGG	7480	TTTATGCGGG	7490	GGATAACTGC	7500	AGTTACTGCC
7510	TAAATGCCCC	7520	GCCTGGCAAT	7530	ATGCCCAGTA	7540	CATGACCTTA	7550	TGGGACTTTC	7560	CTACTTGGCA
7510	ATTACCGGG	7520	CGGACCGTAA	7530	TACGGGTCTAT	7540	GTACTGTGAAT	7550	ACCTGTAAAG	7560	GATGAACCGT
7570	GTACATCTAC	7580	GTATTAGTCA	7590	TCGCTATTAC	7600	CATGTTGATG	7610	CGGTTTGGC	7620	AGTACATCAA
7570	CATGTAGATG	7580	CATAATCAGT	7590	AGCGATAATG	7600	GTACCACTAC	7610	GCCAAAACCG	7620	TCATGTAGTT
7630	TGGCGGTGGA	7640	TAGCGGTTTG	7650	ACTCACGGGG	7660	ATTTCCAGT	7670	CTCCACCCCA	7680	TTGACGTCAA
7630	ACCCGCACCT	7640	ATCGCCAAAC	7650	TGAGTGCCCC	7660	TAAAGGTTCA	7670	GAGGTGGGGT	7680	AACTGCAGTT
7690	TGGGAGTTTG	7700	TTTTGGCACC	7710	AAAATCAACG	7720	GGACTTTCCA	7730	AAATGTCTGA	7740	ACAACTCCGC
7690	ACCCCTAAAC	7700	AAAACCCGTG	7710	TTTTTAGTTTC	7720	CCTGAAAGGT	7730	TTTACAGCAT	7740	TGTTGAGGGG
7750	CCCATTGACG	7760	CAAATGGGCG	7770	GTAGGGGTGT	7780	ACGGTGGGAG	7790	GTCTATATAA	7800	GCAGAGCTCT
7750	GGGTAACTGC	7760	GTTTACCCGC	7770	CATCCGACACA	7780	TGCCACCCCTC	7790	CAGATATAAT	7800	CGTCTCGAGA

FIG. 19N

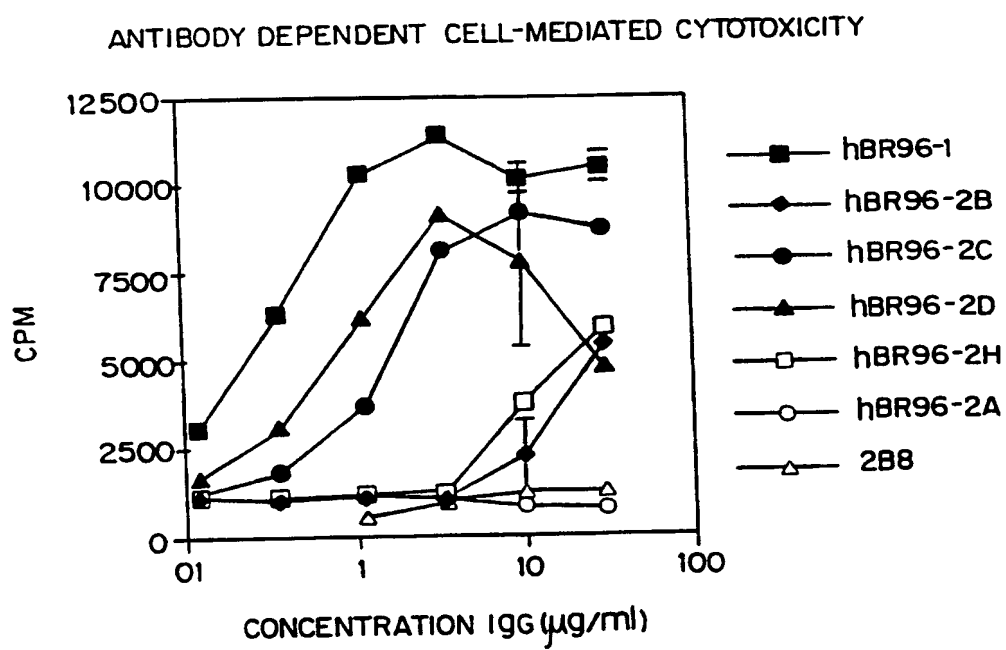
pD17-hG1b

7810	CTGGCTAACT	7820	AGAGAACCCA	7830	CTGCTTACTG	7840	GCTTATCGAA	7850	ATTAATACGA	7860	CTCACTATAG
	GACCGATTGA		TCTCTTGGGT		GACGAATGAC		CGAATAGCTT		TAATTATGCT		GAGTGATATC
7870	GGAGACCCAA	7880	GCTT								
	CCTCTGGGTT		CGAA								

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Fig. 20

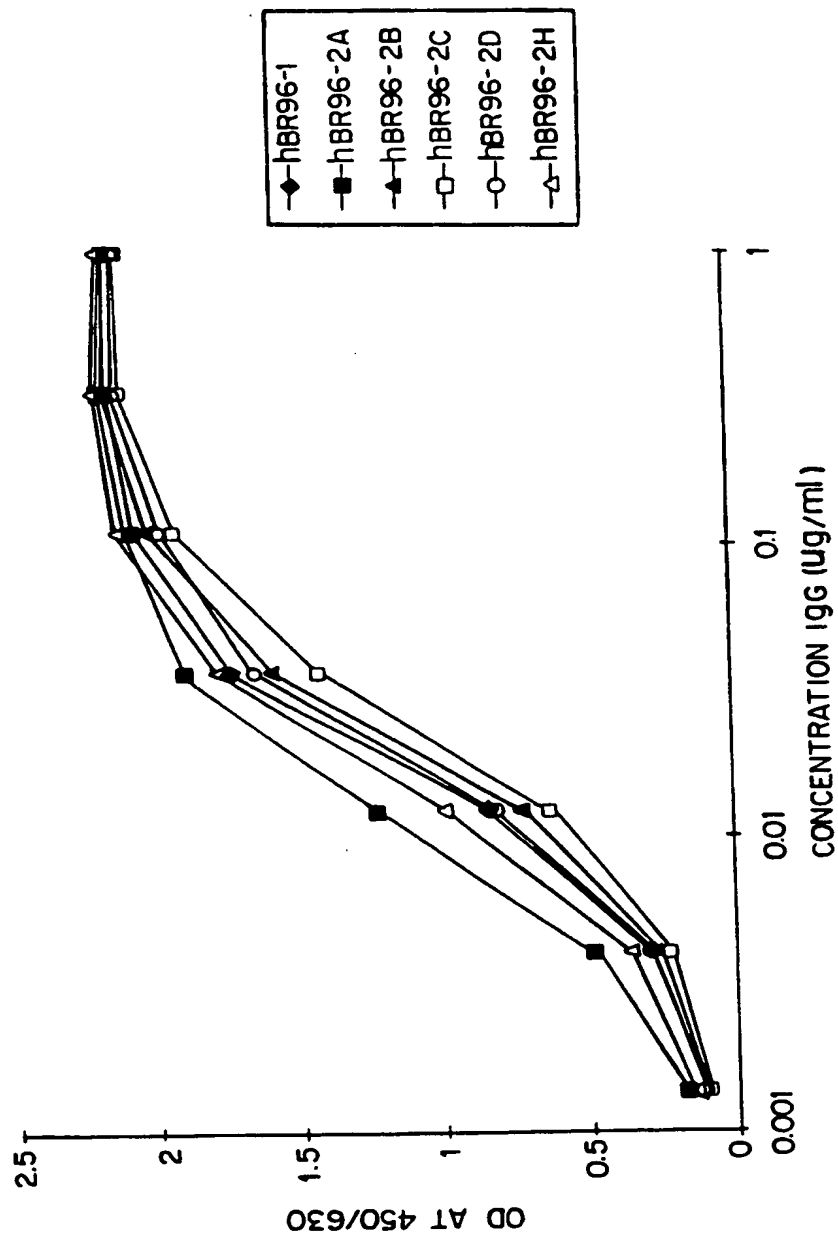
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Fig. 21

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Fig. 22

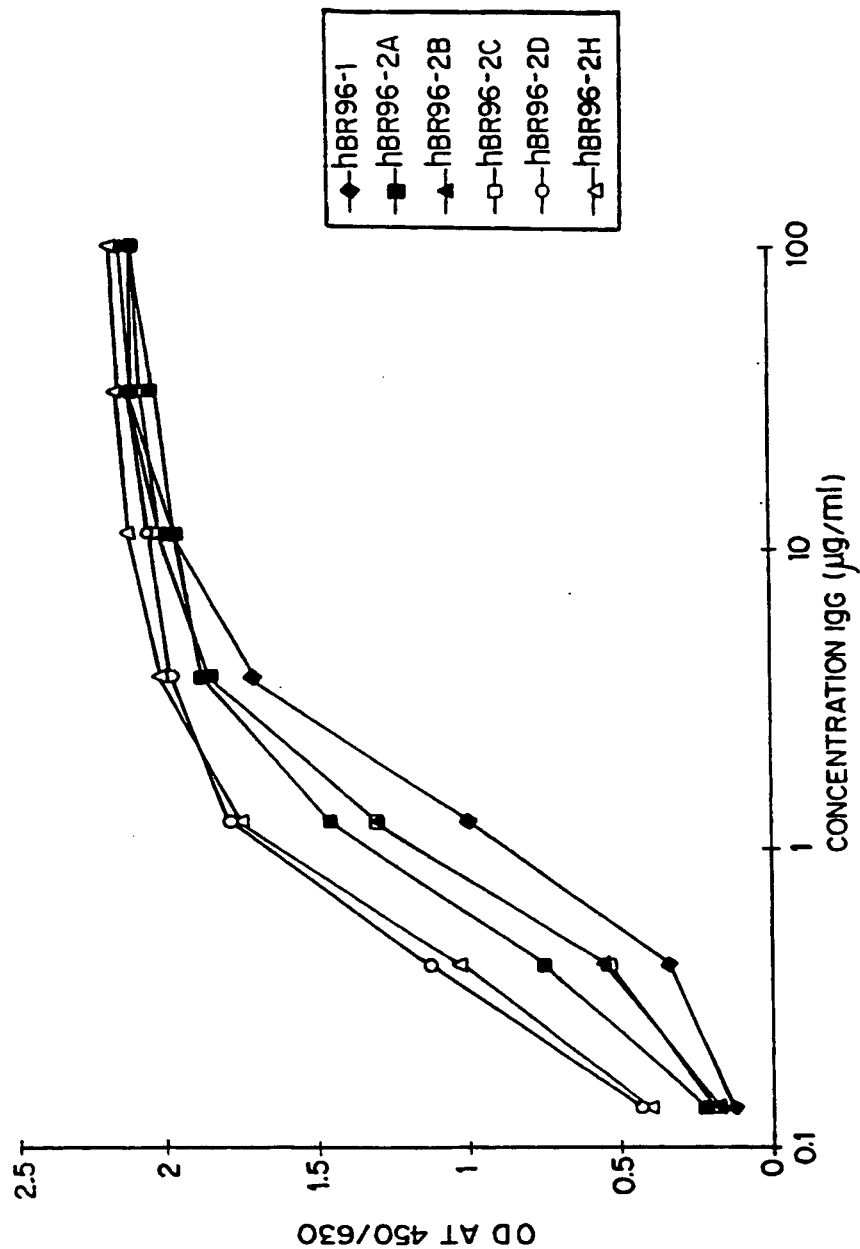
BINDING ACTIVITY OF hBR96-2 CONSTANT REGION MUTANTS ON LEY-HSA



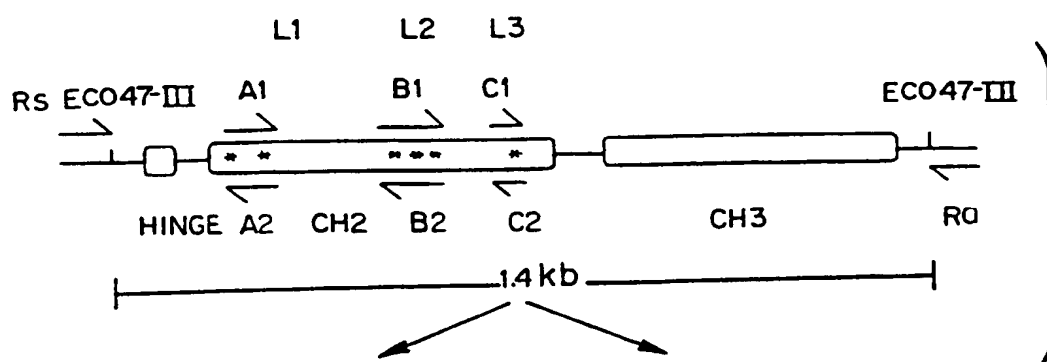
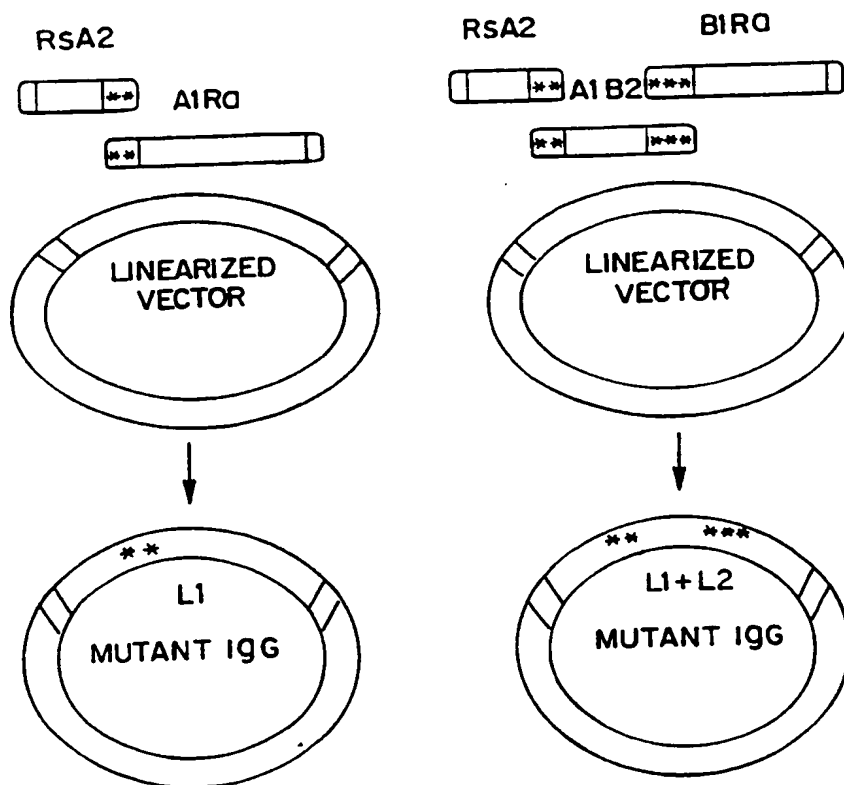
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Fig. 23

BINDING ACTIVITY OF hBR96-2 CONSTANT REGION MUTANTS ON LNFP III-BSA



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Fig. 24A**Fig. 24B**

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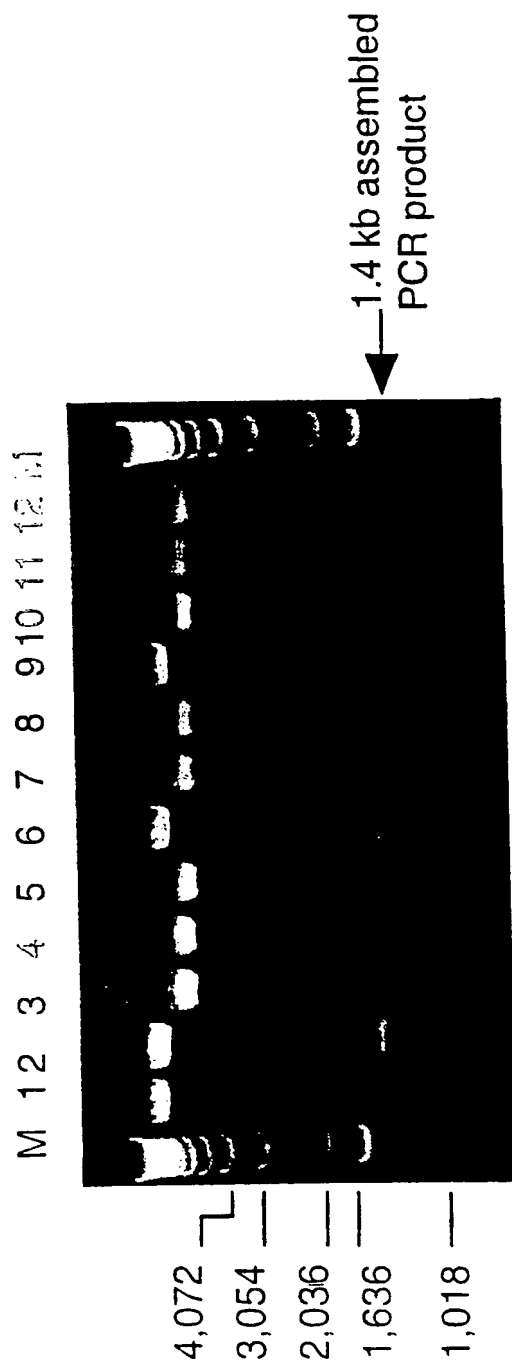


FIG. 25

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FIG. 28

This sequence is the chi BR96 IgG1 with CH2 deleted.

VH
1 EVNLVESGGG LVQPGGSLKV SCVTSGFTFS DYMYWVRQT PEKRLEWVAY
51 ISQGGDITDY PDTVKGRFTI SRDNAKNTLY LQMSRLKSED TAMYVCARGL
CH1
101 DDGAWFAYWG QGTLVTVSVA STKGPSVFPL APSSKSTSGG TAALGCLVKD
151 YFPEPVTVSW NSGALTSGVH TFP AVLQSSG LYSLSVVTV PSSSLGTQTY
CH3
201 ICNVN HKPSN TKVDKKVEPK SCDKTHTCPP CPGQPREPQV YTLPPSRDEL
251 TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTPPV L DSDGSFFLYS
301 KLTVDKSRWQ QGNVFSCSVH HEALHNHYTQ KSLSLSPGK

INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/US 97/13562

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/62 A61K39/395 A61K38/17 A61K47/48 A61K51/10
C07K16/30 C07K16/46 C07K16/00 C12N15/13 C12N1/21
C12N5/10 //C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. GILLIES ET AL.: "Antigen binding and biological activities of engineered mutant chimeric antibodies with human tumor specificities." HUMAN ANTIBODIES AND HYBRIDOMAS, vol. 1, no. 1, 1990, STONEHAM, MA, USA, pages 47-54, XP002050448 see the whole document --- -/-	1-8, 23-25

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

17 December 1997

Date of mailing of the international search report

21.01.98

Name and mailing address of the ISA

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Authorized officer

Nooij, F

FIG. 26

hBR96-2 Heavy Chain Variable Region (V_H)

```

1           11           21           31           41
EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYMYWVRQA PGKGLEWVSY
51          61          71          81          91
ISQDGDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL
101         111
ADGAWFAYWG QGTLVTVSS

```

Human IgG1 Constant

```

CH1
A STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CH2 235 237
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKDKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

```

FIG. 27**hBR96-2A: Heavy Chain Variable Region (V_H)**

1 11 21 31 41
EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYMYWVRQA PGKGLEWVSY
51 61 71 81 91
ISQDGDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL
101 111
ADGAWFAYWG QGTLVTVSS

hBR96-2A: Human Heavy Chain IgG1 Constant Region ΔCH2

A STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH
TFPAVLQSSG LYSLSVVTV PSSSLGTQTY ICNVNHNKPSN TKVDKKVEPK
SCDKTHTCPP CP GQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA
VEWESNGQPE NNYKTTTPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
HEALHNHYTQ KSLSLSPGK

INTERNATIONAL SEARCH REPORT

Intern. 1al Application No

PCT/US 97/13562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. SCHREIBER ET AL.: "An unmodified anticarcinoma antibody, BR96, localizes to and inhibits the outgrowth of human tumors in nude mice." CANCER RESEARCH, vol. 52, no. 12, 15 June 1992, BALTIMORE, MD, USA, pages 3262-3266, XP002050449 see abstract	33,35,36
A	---	1,2,5,7, 8,11-18, 23
A	A. DUNCAN ET AL.: "The binding site for Clq on IgG." NATURE, vol. 332, no. 6166, 21 April 1988, LONDON, GB, pages 738-740, XP002050450 cited in the application see the whole document	1,2,5,7, 8
A	---	1,2,5,7, 8
A	J. LUND ET AL.: "Human FcγRI and FcγRII interact with distinct but overlapping sites on human IgG." THE JOURNAL OF IMMUNOLOGY, vol. 147, no. 8, 15 October 1991, BALTIMORE, MD, USA, pages 2657-2662, XP002050451 cited in the application see abstract	1,2,5,7, 8
A	---	1-8
A	Y. XU ET AL.: "Residue at position 331 in the IgG1 and IgG4 CH2 domains contributes to their differential ability to bind and activate complement." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 5, 4 February 1994, BALTIMORE, MD, USA, pages 3469-3474, XP002050452 cited in the application see abstract see discussion	1,2,5,7, 8
A	---	1,2,5,7, 8
	T. MICHAELSEN ET AL.: "One disulfide bond in front of the second heavy chain constant region is necessary and sufficient for effector functions of human IgG3 without a genetic hinge." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 91, no. 20, 27 September 1994, WASHINGTON, DC, USA, pages 9243-9247, XP002050453 see the whole document	1,2,5,7, 8

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INTERNATIONAL SEARCH REPORT

Intern. Application No.
PCT/US 97/13562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	L. TAN ET AL.: "Influence of the hinge region on complement activation, C1q binding, and segmental flexibility in chimeric human immunoglobulins." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 87, no. 1, January 1990, WASHINGTON, DC, USA, pages 162-166, XP002050454 see the whole document ---	1-8
A	EP 0 699 756 A (BRISTOL-MYERS SQUIBB COMPANY) 6 March 1996 cited in the application see examples see claims -----	11-18, 23,25, 28,29, 31-52

INTERNATIONAL SEARCH REPORT

International application No
PCT/US 97/13562

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/13562

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 26,27

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 26 represents a method of detection/diagnosis and refers forward to claim 30, which represents a method of treatment. Claim 27 refers to a method in claim 24; however, in claim 24 a product is claimed, not a method.

Remark : Although claims 1-22, 25, 28-32 and 34-36 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/13562

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 699756 A	06-03-96	AU 2834995 A	15-02-96
		CA 2155397 A	05-02-96
		JP 8191692 A	30-07-96
